ACTIVATED T CELLS, NERVOUS SYSTEM-SPECIFIC ANTIGENS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of application Serial No. 09/314,161, filed May 19, 1999, which is a continuation-in-part of application No. PCT/US98/14715, filed July 21, 1998, and is a continuation-in-part of application Serial No. 09/218,277, filed December 22, 1998, the entire contents of each of which are hereby incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions and methods for the promotion of nerve regeneration or prevention or inhibition of neuronal degeneration to ameliorate the effects of injury or disease of the nervous system (NS). In the certain embodiments, activated T cells, an NS-specific antigen or peptide derived therefrom or a nucleotide sequence encoding an NS-specific antigen can be used to promote nerve regeneration or to confer neuroprotection and prevent or inhibit neuronal degeneration caused by injury or disease of nerves within the central nervous system or peripheral nervous system of a human subject. The compositions of the present invention may be administered alone or may be optionally administered in any desired combination.

[0003] **Abbreviations**: **APC**: antigen-presenting cell; **BSA**: bovine serum albumin; **CAP**: compound action potential; **CFA**: complete Freund's adjuvant; **CNS**: central nervous system; **4-Di-10-Asp**: 4-(4-didecylamino)styryl)-N-methylpyridinium iodide; **EAE**: experimental autoimmune encephalomyelitis; **FCS**: fetal calf serum; **IFA**: incomplete Freund's adjuvant **MAG**: myelin-associated glycoprotein; **MBP**: myelin basic protein; **MOG**: myelin oligodendrocyte glycoprotein; **NS**: nervous system; **OVA**: ovalbumin; **PBS**: phosphate-buffered saline; **PLP**: proteolipid protein; **PNS**: peripheral nervous system; **RGC**: retinal ganglion cells; **TCR**: T-cell receptor.

BACKGROUND OF THE INVENTION

[0004] The nervous system comprises the central (CNS) and the peripheral (PNS) nervous system. The CNS is composed of the brain, the spinal cord and the visual system; the PNS consists of all of the other neural elements, namely the nerves and ganglia outside of the brain and spinal cord.

[0005] Damage to the nervous system may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease or disorder including, but not limited to Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis (ALS), diabetic neuropathy, senile dementia, stroke and ischemia.

[0006] Maintenance of CNS integrity is a complex balancing act in which compromises are struck with the immune system. In most tissues, the immune system plays an essential part in protection, repair, and healing. In the CNS, because of its unique immune privilege, immunological reactions are relatively limited (Streilein, 1993). A growing body of evidence indicates that the failure of the mammalian CNS to achieve functional recovery after injury is a reflection of an ineffective dialog between the damaged tissue and the immune system. For example, the restricted communication between the CNS and blood-borne macrophages affects the capacity of axotomized axons to regrow; transplants of activated macrophages can promote CNS regrowth (Lazarov-Spiegler et al, 1996; Rapalino et al, 1998).

[0007] Activated T cells have been shown to enter the CNS parenchyma, irrespective of their antigen specificity, but only T cells capable of reacting with a CNS antigen seem to persist there (Hickey et al, 1991; Werkele, 1993; Kramer et al, 1995). T cells reactive to antigens of CNS white matter, such as myelin basic protein (MBP), can induce the paralytic disease experimental autoimmune encephalomyelitis (EAE) within several days of their inoculation into naive recipient rats (Ben-Nun et al, 1981a). Anti-MPB T cells may also be involved in the human disease multiple sclerosis (Ota et al, 1990;

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Martin, 1997). However, despite their pathogenic potential, anti-MBP T cell clones are present in the immune systems of healthy subjects (Burns et al, 1983; Pette et al, 1990; Martin et al, 1990; Schluesener et al, 1985). Activated T cells,

5 which normally patrol the intact CNS, transiently accumulate at sites of CNS white matter lesions (Hirschberg et al, 1998).

[0008] A catastrophic consequence of CNS injury is that the primary damage is often compounded by the gradual secondary loss of adjacent neurons that apparently were undamaged, or

10 only marginally damaged, by the initial injury (Faden et al, 1992; Faden, 1993; McIntosh, 1993). The primary lesion causes changes in extracellular ion concentrations, elevation of amounts of free radicals, release of neurotransmitters, depletion of growth factors, and local inflammation. These

15 changes trigger a cascade of destructive events in the adjacent neurons that initially escaped the primary injury (Lynch et al, 1994; Bazan et al, 1995; Wu et al, 1994). This secondary damage is mediated by activation of voltage-dependent or agonist-gated channels, ion leaks, activation of

20 calcium-dependent enzymes such as proteases, lipases and nucleases, mitochondrial dysfunction and energy depletion, culminating in neuronal cell death (Yoshina et al, 1991; Hovda et al, 1991; Zivin et al, 1991; Yoles et al, 1992). The

widespread loss of neurons beyond the loss caused directly by the primary injury has been called "secondary degeneration."

[0009] Another tragic consequence of CNS injury is that neurons in the mammalian CNS do not undergo spontaneous regeneration following an injury. Thus, a CNS injury causes permanent impairment of motor and sensory functions.

[0010] Spinal cord lesions, regardless of the severity of the injury, initially result in a complete functional paralysis known as spinal shock. Some spontaneous recovery from spinal shock may be observed, starting a few days after the injury and tapering off within three to four weeks. The less severe the insult, the better the functional outcome. The extent of recovery is a function of the amount of undamaged tissue minus the loss due to secondary degeneration. Recovery from injury would be improved by neuroprotective treatment that could reduce secondary degeneration.

[0011] The parent applications, application nos. 09/218,277 and 09/314,161 and PCT Publication WO 99/60021, describe the discovery made in the laboratory of the present inventors that activated T cells that recognize an antigen of the NS of the patient confer neuroprotection. More specifically, T cells reactive to MBP were shown to be neuroprotective in rat models of partially crushed optic nerve (see also Moalem et al, 1999a, the entire contents of which being hereby incorporated

herein by reference) and of spinal cord injury (see also

Hauben et al, 2000, the entire contents of which being hereby

incorporated herein by reference). Until recently, it had

been thought that immune cells do not participate in NS

5   repair. Furthermore, any immune activity in the context of

CNS damage was traditionally considered detrimental for

recovery. It was quite surprising to discover that NS-

specific activated T cells could be used to protect nervous

system tissue from secondary degeneration which may follow

10 damage caused by injury or disease of the CNS or PNS. The

mechanism of action of such NS-specific T cells has yet to be

discovered, but the massive accumulation of exogenously

administered T cells at the site of CNS injury suggests that

the presence of T cells at the site of injury plays a

15 prominent role in neuroprotection. It appears, however, that

the accumulation, though a necessary condition, is not

sufficient for the purpose, as T cells specific to the non-

self antigen ovalbumin also accumulate at the site, but have

no neuroprotective effect (Hirschberg et al, 1998).

20 [0012] In addition to the NS-specific activated T cells,

the above-referenced US applications and PCT publication WO

99/60021 disclose that therapy for amelioration of effects of

injury or disease of NS can be carried out also with a natural

or synthetic NS-specific antigen such as MAG, S-100, β-

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amyloid, Thy-1, P0, P2, a neurotransmitter receptor, and preferably human MBP, human proteolipid protein (PLP), and human oligodendrocyte glycoprotein (MOG), or with a peptide derived from an NS-specific antigen such as a peptide

5   comprising amino acids 51-70 of MBP or amino acids 35-55 of MOG.

[0013] Citation or identification of any reference in this section or any other part of this application shall not be construed as an admission that such reference is available as

10   prior art to the invention.

SUMMARY OF THE INVENTION

[0014] The present invention is directed to methods and compositions for promotion of nerve regeneration or for neuroprotection and prevention or inhibition of neuronal

15   degeneration to ameliorate the effects of injury to, or disease of, the nervous system (NS).

[0015] The present invention is based in part on the inventors' unexpected discovery that activated T cells that recognize an antigen of the NS of the patient promote nerve

20   regeneration or confer neuroprotection. As used herein, "neuroprotection" refers to the prevention or inhibition of degenerative effects of injury or disease in the NS. Since it was thought until recently that immune cells do not participate in nervous system repair, it was quite surprising

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to discover that NS-specific activated T cells and also the
NS-specific antigens themselves and peptides derived therefrom
can be used to promote nerve regeneration or to protect
nervous system tissue from secondary degeneration which may
5   follow damage caused by injury or disease of the CNS or PNS.

[0016] Thus, in one aspect, the invention relates to a
method for promoting nerve regeneration or for conferring
neuroprotection and preventing or inhibiting neuronal
degeneration in the central nervous system or peripheral
10   nervous system for ameliorating the effects of injury or
disease, comprising administering to an individual in need
thereof at least one ingredient selected from the group
consisting of:

[0017] (a)   NS-specific activated T cells;

15   [0018] (b)   a NS-specific antigen or an analog thereof;

[0019] (c)   a peptide derived from an NS-specific antigen
or from an analog thereof, or an analog or derivative of said
peptide;

[0020] (d)   a nucleotide sequence encoding an NS-specific
20   antigen or an analog thereof;

[0021] (e)   a nucleotide sequence encoding a peptide
derived from an NS-specific antigen or from an analog thereof,
or an analog of said peptide; or

[0022] (f)   any combination of (a)-(e).

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[0023] In another aspect, the invention relates to a pharmaceutical composition for promoting nerve regeneration or for neuroprotection and prevention or inhibition of neuronal degeneration in the CNS or PNS for ameliorating the effects of injury or disease in the NS, comprising a therapeutically effective amount of at least one ingredient selected from the group consisting of (a) to (e) above or any combination of (a)-(e).

[0024] The term "NS-specific antigen" as used herein refers to an antigen of the NS that specifically activates T cells such that following activation the activated T cells accumulate at a site of injury or disease in the NS of the patient. Examples of NS-specific antigens according to the invention include, but are not limited to, myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), S-100, β-amyloid, Thy-1, P0, P2, neurotransmitter receptors, the protein Nogo (Nogo-A, Nogo-B and Nogo-C) and the Nogo receptor (NgR). This definition also includes analogs of said NS-specific antigens as described in the section on NS-specific antigens, analogs thereof, peptides derived therefrom and analogs and derivatives thereof of said peptides hereinafter.

[0025] Fig. 1 is a bar graph showing the presence of T

cells in uninjured optic nerve or in injured optic nerve one

week after injury. Adult Lewis rats were injected with

5   activated T cells of the anti-MBP (T_{MBP}), anti-OVA (T_{OVA}), anti-

p277 (a peptide of the human hsp60) (T_{p277}) lines, or with PBS,

immediately after unilateral crush injury of the optic nerve.

Seven days later, both the injured and uninjured optic nerves

were removed, cryosectioned and analyzed immunohistochemically

10   for the presence of immunolabeled T cells. T cells were

counted at the site of injury and at randomly selected areas

in the uninjured optic nerves. The histogram shows the mean

number of T cells per mm^2 ± s.e.m., counted in two to three

sections of each nerve. Each group contained three to four

15   rats. The number of T cells was considerably higher in

injured nerves of rats injected with anti-MBP, anti-OVA or

anti-p277 T cells; statistical analysis (one-way ANOVA) showed

significant differences between T cell numbers in injured

optic nerves of rats injected with anti-MBP, anti-OVA, or

20   anti-p277 T cells and the T cell numbers in injured optic

nerves of rats injected with PBS ($P<0.001$); and between

injured optic nerves and uninjured optic nerves of rats

injected with anti-MBP, anti-OVA, or anti-p277 T cells

($P<0.001$).

[0026] Fig. 2 is a bar graph illustrating that T cells specific to MBP, but not to OVA or p277, protect neurons from secondary degeneration. Immediately after optic nerve injury, rats were injected with anti-MBP, anti-OVA or anti-p277 T cells, or with PBS. The neurotracer dye 4-Di-10-Asp was applied to optic nerves distal to the site of the injury, immediately after injury (for assessment of primary damage) or two weeks later (for assessment of secondary degeneration). Five days after dye application, the retinas were excised and flat-mounted. Labeled retinal ganglion cells (RGCs) from three to five randomly selected fields in each retina (all located at approximately the same distance from the optic disk) were counted by fluorescence microscopy. RGC survival in each group of injured nerves was expressed as the percentage of the total number of neurons spared after the primary injury (42% of neurons remained undamaged after the primary injury). The neuroprotective effect of anti-MBP T cells compared with that of PBS was significant (P<0.001, one-way ANOVA). Anti-OVA T cells or anti-p277 T cells did not differ significantly from PBS in their effects on the protection of neurons that had escaped primary injury (P>0.05, one-way ANOVA). The results are a summary of five experiments. Each group contained five to ten rats.

[0027] Figs. 3 (A-C) present photomicrographs of retrogradely labeled retinas of injured optic nerves of rats. Immediately after unilateral crush injury of their optic nerves, rats were injected with PBS (Fig. 3A) or with activated anti-p277 T cells (Fig. 3B) or activated anti-MBP T cells (Fig. 3C). Two weeks later, the neurotracer dye 4-Di-10-Asp was applied to the optic nerves, distal to the site of injury. After 5 days, the retinas were excised and flat-mounted. Labeled (surviving) RGCs, located at approximately the same distance from the optic disk in each retina, were photographed.

[0028] Figs. 4(A-B) are graphs showing that clinical severity of EAE is not influenced by an optic nerve crush injury. For the results presented in Fig. 4A, Lewis rats, either uninjured (dash line) or immediately after optic nerve crush injury (solid line), were injected with activated anti-MBP T cells. EAE was evaluated according to a neurological paralysis scale. [Data points represent ± s.e.m.] These results represent a summary of three experiments. Each group contained five to nine rats. Fig. 4B shows that the number of RGCs in the uninjured optic nerve is not influenced by injection of anti-MBP T cells. Two weeks after the injection of anti-MBP T cells or PBS, 4-Di-10-Asp was applied to the optic nerves. After 5 days the retinas were excised and-flat-

mounted. Labeled RGCs from five fields (located at approximately the same distance from the optic disk) in each retina were counted and the average number per mm^2 was calculated. There was no difference between the numbers of

5   labeled RGCs in rats injected with anti-MBP T cells (T_{MBP}) and in PBS-injected control rats.

[0029] Fig. 5 is a bar graph showing that T cells specific to p51-70 of MBP protect neurons from secondary degeneration. Immediately after optic nerve injury, rats were injected with

10   anti-MBP T cells, anti-p51-70 T cells, or PBS. 4-Di-10-Asp was applied to optic nerves distal to the site of the injury, immediately after injury (for assessment of primary damage) or two weeks later (for assessment of secondary degeneration). Five days after dye application, the retinas were excised and

15   flat-mounted. Labeled RGCs from three to five randomly selected fields in each retina (all located at approximately the same distance from the optic disk) were counted by fluorescence microscopy. RGC survival in each group of injured nerves was expressed as the percentage of the total

20   number of neurons spared after primary injury. Compared with that of PBS treatment, the neuroprotective effects of anti-MBP and of anti-p51-70 T cells were significant (P<0.001, one-way ANOVA).

[0030] Figs. 6(A-B) are graphs showing that anti-MBP T cells increase the compound action potential (CAP) amplitudes of injured optic nerves. Immediately after optic nerve injury, rats were injected with either PBS or activated anti-MBP T cells (T_{MBP}). Two weeks later, the CAPs of injured (Fig. 6A) and uninjured (Fig. 6B) nerves were recorded. There were no significant differences in mean CAP amplitudes between uninjured nerves obtained from PBS-injected and anti-MBP T cell-injected rats (n-8; p=0.8, Student's t-test). The neuroprotective effect of anti-MBP T cells (relative to PBS) on the injured nerve on day 14 after injury was significant (n=8, p=0.009, Student's t-test).

[0031] Figs. 7(A-B) are graphs showing recovery of voluntary motor activity as a function of time after contusion, with and without injection of autoimmune anti-MBP T cells. (Fig. 7A) Twelve rats were deeply anesthetized and laminectomized, and then subjected to a contusion insult produced by a 10 gram weight dropped from a height of 50 mm. Six of the rats, selected at random, were then inoculated i.p. with 10^7 anti-MBP T cells and the other six were inoculated with PBS. At the indicated time points, locomotor behavior in an open field was scored by observers blinded to the treatment received by the rats. Results are expressed as the mean values for each group. The vertical bars indicate SEM.

Differences tested by repeated ANOVA, including all time

points, were significant (p<0.05). (Fig. 7B) A similar

experiment using five PBS-treated animals and six animals

treated with anti-MBP T cells were all subjected to a more

5   severe contusion. At the indicated time points, locomotor

behavior in an open field was scored. The results are

expressed as the mean values for each group. The vertical

bars indicate SEM. Rats in the treated group are represented

by open circles and rats in the control group are represented

10   by black circles. Horizontal bars show the median values.

The inset shows the median plateau values of the two groups.

[0032] Figs. 8(A-C) show retrograde labeling of cell bodies

at the red nucleus in rats treated with autoimmune anti-MBP T

cells (8A) and in control injured (8B) rats. Three months

15   after contusion and treatment with anti-MBP T cells, some rats

from both the treated and the control groups were re-

anesthetized and a dye was applied below the site of the

contusion. After five to seven days, the rats were again

deeply anesthetized and their brains were excised, processed,

20   and cryosectioned. Sections taken through the red nucleus

were inspected and analyzed qualitatively and quantitatively

under fluorescent and confocal microscopes. Significantly,

more labeled nuclei were seen in the red nuclei of rats

treated with anti-MBP T cells (8A) than in the red nuclei of

PBS-treated rats (8B). The quantitative differences are shown

in the bar graph (8C) and were obtained from animals with

scores of 10 and 11 in the T-cell-treated group and scores of

6 in the control group. The bar graph shows mean ± SD.

5   [0033] Fig. 9 is a series of photographs showing diffusion-

weighted imaging of contused spinal cord treated with anti-MBP

T cells. Spinal cords of MBP-T cell-treated and PBS-treated

animals (with locomotion scores of 10 and 8, respectively)

were excised under deep anesthesia, immediately fixed in 4%

10   paraformaldehyde solution, and placed into 5 mm NMR tubes.

Diffusion anisotropy was measured in a Bruker DMX 400 widebore

spectrometer using a microscopy probe with a 5-mm Helmholtz

coil and actively shielded magnetic field gradients. A

multislice pulsed gradient spin echo experiment was performed

15   with 9 axial slices, with the central slice positioned at the

center of the spinal injury. Images were acquired with TE of

31 ms, TR of 2000 ms, a diffusion time of 15 ms, a diffusion

gradient duration of 3 ms, field of view 0.6 mm, matrix size

128 x 128, slice thickness 0.5 mm, and slice separation of

20   1.18 mm. Four diffusion gradient values of 0, 28, 49, and 71

g/cm were applied along the read direction (transverse

diffusion) or along the slice direction (longitudinal

diffusion). Diffusion anisotropy is manifested by increased

signal intensity in the images with the highest transverse

diffusion gradient relative to the longitudinal diffusion gradient. The excised spinal cords of a PBS-treated rat and in the rat treated with MBP-T cells were subjected to diffusion-weighted MRI analysis. In the PBS-treated injured control, diffusion anisotropy was seen mainly in sections near the proximal and distal stumps of the cord, with low anisotropy in sections taken through the site of injury. In contrast, in the treated rat, higher levels of diffusion anisotropy can be seen in sections taken through the site of injury.

[0034] Fig. 10 is a graph illustrating inhibition of secondary degeneration after optic nerve crush injury in adult rats. See text, Example 3, for experimental details. Rats were injected intradermally through the footpads with a 21-mer peptide based on MOG amino acid residues 35-55 (MOG p35-55) ((50 μ/animal, chemically synthesized at the Weizmann Institute of Science, Rehovot, Israel) or PBS ten days prior to optic nerve crush injury or MOG p35-55 in the absence of crush injury. MOG p35-55 was administered with IFA. Surviving optic nerve fibers were monitored by retrograde labeling of RGCs. The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

[0035] Fig. 11 is a graph illustrating inhibition in adult rats of secondary degeneration after optic nerve crush injury by MBP. See text, Example 4, for experimental details. MBP (Sigma, Israel) (1 mg in 0.5 ml saline) was administered orally to adult rats by gavage using a blunt needle. MBP was administered 5 times, i.e., every third day beginning two weeks prior to optic nerve crush injury. Surviving optic nerve fibers were monitored by retrograde labeling of RGCs. The number of RGCs in treated rats was expressed as a percentage of the total number of neurons in untreated rats following the injury.

[0036] Figs. 12 (A-F) show expression of B7 co-stimulatory molecules in intact and injured rat optic nerve. Optic nerves were excised from adult Lewis rats before (12A, 12B) and three days after injury (12C, 12D, 12E) and analyzed immunohistochemically for expression of the B7 co-stimulatory molecule. The site of injury was delineated by GFAP staining. Using calibrated cross-action forceps, the right optic nerve was subjected to a mild crush injury 1-2 mm from the eye. The uninjured cointralateral nerve was left undisturbed. Immunohistochemical analysis of optic nerve antigens was performed as follows. Briefly, longitudinal cryosections of the excised nerves (20 μm thick) were picked up onto gelatin-coated glass and fixed with ethanol for ten minutes at room

temperature. The sections were washed and incubated for one
hour at room temperature with mouse monoclonal antibody to rat
GFAP (BioMakor, Israel), diluted 1:100, and with antibodies to
B7.2 co-stimulatory molecule and the B7.1 co-stimulatory

5   molecule (Pharmingen, San Diego, CA), diluted 1:25. The
sections were washed again and incubated with rhodamine
isothiocyanate-conjugated goat anti-mouse IgG (with minimal
cross-reaction to rat, human, bovine and horse serum protein)
(Jackson ImmunoResearch, West Grove, PA), for one hour at room

10 temperature. All washing solutions contained PBS and 0.05%
Tween-20. All diluting solutions contained PBS containing 3%
fetal calf serum and 2% bovine serum albumin. The sections
were treated with glycerol containing 1,4-diazobicyclo-
(2,2,2)-octane and were then viewed with a Zeiss microscope.

15 Note the morphological changes of the B7.2 positive cells
after injury, from a rounded (12A, 12B) to a star-like shape
(12C, 12D). The B7.2 positive cells were present at a higher
density closer to the injury site (12E). Expression of B7.1
was detectable only from day seven and only at the injured

20 site (12F).

[0037] Figs. 13 A-C show immunohistochemical analysis of T
cells, macrophages or microglia, and B7.2 co-stimulatory
molecules in the injured optic nerves of rats fed MBP. Lewis
rats aged 6-8 weeks were fed 1 mg of bovine MBP (Sigma,

Israel) (2 mg MBP/ml PBS) or 0.5 ml PBS only every other day

by gastric intubation using a stainless steel feeding needle

(Thomas Scientific, Swedesboro, NJ) (Chen et al, 1994). Ten

days after starting MBP, the right optic nerves were subjected

5   to calibrated crush injury, as described for Fig. 12. Three

days later, the nerves were excised and prepared for

immunohistochemical analysis of T cells using mouse monoclonal

antibodies to T cell receptor 11, diluted 1:25, macrophages or

microglia using anti-ED1 antibodies (Serotek, Oxford, U.K)

10 diluted 1:250, astrocytes using anti-GFAP antibodies and B7.2

co-stimulatory molecules as described for Fig. 12. There were

no significant quantitative differences in T cells or in ED-1

positive cells between injured optic nerves of PBS-fed (13A)

and MBP-fed (13B) rats. The number of B7.2 positive cells at

15 the site of injury of MBP-fed rats (13C) should be noted, as

compared with injured controls (see Fig. 12E above).

[0038] Fig. 14 is a graph showing the slowing of neuronal

degeneration in rats with orally induced tolerance to MBP.

Lewis rats were fed 1 mg MBP daily, or every other day, or 4

20 times a day at two-hour intervals for five consecutive days.

Control animals were given PBS or the non-self antigen OVA

Sigma, Israel). Ten days after the start of MBP ingestion,

the right optic nerves were subjected to a calibrated mild

crush injury. Two weeks later the RGCs were retrogradely

labelled by application of the fluorescent lipophilic dye 4-
Di-10-Asp(Molecular Probes Europe BV, Netherlands), distally
to the site of injury, as described. Briefly, complete
axotomy was performed 1-2 mm from the distal border to the
injury site, and solid crystals (0.2-0.4 mm in diameter) of 4-
Di-10-Asp were immediately deposited at the site of the
lesion. Retrograde labeling of RGCs by the dye gives a
reliable indication of the number of still-functioning
neurons, as only intact axons can transport the dye to their
cell bodies in the retina. Six days after dye application,
the retina was detached from the eye, prepared as a flattened
whole mount in 4% paraformaldehyde solution, and examined for
labeled RGCs by fluorescence microscopy. RGCs were counted
from three different regions in the retina. The results are
expressed as normalized percentage of each retina to untreated
injured animal mean of the same experiment. The median of
each group is shown as a bar (Control vs. MBP OTx4 ** P<0.01;
Control vs. MBP OT ** P, 0.01; Control vs. OVA OT ns P>0.05.

[0039] Fig. 15 shows the nucleotide sequence of rat MBP
gene, SEQ ID NO:1, Genbank accession number M25889 (Schaich et
al, 1986).

[0040] Fig. 16 shows the nucleotide sequence of human MBP
gene, SEQ ID NO:2, Genbank accession number M13577 (Kamholz et
al, 1986).

[0041] Figs 17 (A-F) show the nucleotide sequences of human PLP gene exons 1-7, SEQ ID NOs:3-8, respectively, Genbank accession numbers M15026-M15032, respectively (Diehl et al, 1986).

5   [0042] Fig. 18 shows the nucleotide sequence of human MOG gene, SEQ ID NO:9, Genbank accession number Z48051 (Roth et al, submitted (17-Jan-1995) Roth, CNRS UPR 8291, CIGH, CHU Purpan, Toulouse, France, 31300; Gonzalez et al, 1996).

[0043] Fig. 19 shows the nucleotide sequence of rat PLP gene and variant, SEQ ID NO:10, Genbank accession number M16471 (Nave et al, 1987).

[0044] Fig. 20 shows the nucleotide sequence of rat MAG gene, SEQ ID NO:11, Genbank accession number M14871 (Arquint et al, 1987).

15   [0045] Fig. 21 shows the amino acid sequence of human MBP, SEQ ID NO:12, Genbank accession number 307160 (Kamholz et al, 1986).

[0046] Fig. 22 shows the amino acid sequence of human PLP, SEQ ID NO:13, Genbank accession number 387028.

20   [0047] Fig. 23 shows the amino acid sequence of human MOG, SEQ ID NO:14, Genbank accession number 793839 (Roth et al, 1995; Roth Submitted (17-JAN-1995) Roth CNRS UPR 8291, CIGH, CHU Purpan, Toulouse, France, 31300; Gonzalez et al, 1996).

[0048] Figs. 24 (A-B) show that post-traumatic immunization with Nogo peptide p472 emulsified in CFA promotes functional recovery from spinal cord contusion in comparison to PBS+CFA-treated rats. Spinal cords of male SPD rats were laminectomized at the level of T9 and a 10-g rod was dropped onto the laminectomized cord from a height of 50 mm (Fig. 24A) or of 25 mm (Fig. 24B). See text, Example 5, for experimental details.

[0049] Fig. 25 show that post-traumatic immunization with Nogo peptide p472 emulsified in CFA promotes functional recovery from spinal cord contusion in comparison to PBS-treated or PBS+CFA-treated rats. Spinal cords of female SPD rats were laminectomized at the level of T9 and a 10-g rod was dropped onto the laminectomized cord from a height of 50 mm. See text, Example 5, for experimental details.

DETAILED DESCRIPTION OF THE INVENTION

[0050] As exposed above, the present invention relates to compositions and methods for promoting nerve regeneration or for conferring neuroprotection and preventing or inhibiting neuronal degeneration in the CNS or PNS for ameliorating the effects of injury or disease, comprising administering to an individual in need thereof at least one ingredient selected from the group consisting of:

[0051] (a)   NS-specific activated T cells;

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[0052] (b) a NS-specific antigen or an analog thereof;

[0053] (c) a peptide derived from an NS-specific antigen or from an analog thereof, or an analog or derivative of said peptide;

[0054] (d) a nucleotide sequence encoding an NS-specific antigen or an analog thereof;

[0055] (e) a nucleotide sequence encoding a peptide derived from an NS-specific antigen or from an analog thereof, or an analog of said peptide; or

[0056] (f) any combination of (a)-(e).

[0057] Merely for ease of explanation, the detailed description of the present invention is divided into the following sections: NS-specific activated T cells and T-cell banks; NS-specific antigens, analogs thereof, peptides derived therefrom and analogs and derivatives thereof of said peptides; nucleotide sequences encoding NS-specific antigens, analogs thereof, peptides derived therefrom and analogs thereof; therapeutic uses; and formulations and modes of administration.

NS-SPECIFIC ACTIVATED T CELLS AND T-CELL BANKS

[0058] In one embodiment of the invention, NS-specific activated T cells can be used in an amount which is effective to confer neuroprotection for ameliorating or inhibiting the effects of injury or disease of the CNS or PNS that result in

NS degeneration or for promoting regeneration in the NS, in particular the CNS, as described in the section on therapeutic uses hereinafter.

[0059] In the practice of the invention, administration of NS-specific activated T cells may optionally be in combination with an NS-specific antigen or an analog thereof or a peptide derived therefrom or an analog or derivative of said peptide. Additionally, oral administration of NS-specific antigen or an analog thereof or a peptide derived therefrom or an analog or derivative thereof, can be combined with active immunization to build up a critical T-cell response immediately after injury.

[0060] Activation of T cells is initiated by interaction of a TCR complex with a processed antigenic peptide bound to a MHC molecule on the surface of an antigen-presenting cell (APC). As used herein, the term "activated T cells" includes both (i) T cells that have been activated by exposure to a cognate antigen or peptide derived therefrom or derivative thereof; and (ii) progeny of such activated T cells. As used herein, a "cognate antigen" is an antigen that is specifically recognized by the TCR of a T cell that has been previously exposed to the antigen. Alternatively, the T cell which has been previously exposed to the antigen may be activated by a

mitogen, such as phytohemagglutinin (PHA) or concanavalin A

(Con A).

[0061] The term "NS-specific activated T cell" as used

herein refers to an activated T cell having specificity for an

5   antigen of the NS, said NS-specific antigen being an antigen

of the NS that specifically activates T cells such that these

activated T cells will accumulate at a site of injury or

disease in the NS of the patient. The NS-specific antigen

used to confer the specificity to the T cells may be a self

10 NS-antigen of the patient or a non-self NS-antigen of another

individual or even of another species, or an analog of said

NS-antigen, or a peptide derived from said NS-antigen or from

said analog thereof, or an analog or derivative of said

peptide, all as described in the section on NS-specific

15 antigens, analogs thereof, peptides derived therefrom and

analogs and derivatives thereof of said peptides hereinafter,

as long as the activated T cell recognizes an antigen in the

NS of the patient.

[0062] If the disease being treated by the NS-specific

20 activated T cells of the invention is an autoimmune disease,

in which the autoimmune antigen is an NS antigen, the T cells

which are used in accordance with the present invention for

the treatment of neural damage or degeneration caused by such

disease are preferably not activated against the same

autoimmune antigen involved in the disease. While the prior art has described methods of treating autoimmune diseases by administering activated T cells to create a tolerance to the autoimmune antigen, the T cells of the present invention are

5 not administered in such a way as to create tolerance, but are administered in such a way as to create accumulation of the T cells at the site of injury or disease so as to facilitate neural regeneration or to inhibit neural degeneration.

[0063] The prior art also discloses uses of immunotherapy

10 against tumors, including brain tumors, by administering T cells specific to an NS antigen in the tumor so that such T cells may induce an immune system attack against the tumors. The present invention is not intended to comprehend such prior art techniques. However, the present invention is intended to

15 comprehend the inhibition of neural degeneration or the enhancement of neural regeneration in patients with brain tumors by means other than the prior art immunotherapy of brain tumors. Thus, for example, NS-specific activated T cells, which are activated to an NS-antigen of the patient

20 other than an antigen which is involved in the tumor, would be expected to be useful for the purpose of the present invention and would not have been suggested by known immunotherapy techniques.

[0064] The NS-specific activated T cells are preferably autologous, most preferably of the CD4 and/or CD8 phenotypes, but they may also be semi-allogeneic T cells or allogeneic T cells from related donors, e.g.,, siblings, parents, children, or from donors with the same HLA type (HLA-matched) or a very similar HLA type (HLA-partially matched), or even from unrelated donors.

[0065] Thus, in addition to the use of autologous T cells isolated from the subject, the present invention also comprehends the use of semi-allogeneic T cells for neuroprotection. The T cells may be prepared as short- or long-term lines and stored by conventional cryopreservation methods for thawing and administration, either immediately or after culturing for 1-3 days, to a subject suffering from injury to the CNS and in need of T-cell neuroprotection.

[0066] The use of semi-allogeneic T cells is based on the fact that T cells can recognize a specific antigen epitope presented by foreign APC, provided that the APC expresses the MHC molecule, class I or class II, to which the specific responding T-cell population is restricted, along with the antigen epitope recognized by the T cells. Thus, a semi-allogeneic population of T cells that can recognize at least one allelic product of the subject's MHC molecules, preferably a class II HLA-DR or HLA-DQ or other HLA molecule, and that is

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specific for a NS-associated antigen epitope, will be able to

recognize the NS antigen in the subject's area of NS damage

and produce the needed neuroprotective effect. There is

little or no polymorphism in the adhesion molecules, leukocyte

5   migration molecules, and accessory molecules needed for the T

cells to migrate to the area of damage, accumulate there, and

undergo activation. Thus, the semi-allogeneic T cells will be

able to migrate and accumulate at the CNS site in need of

neuroprotection and will be activated to produce the desired

10 effect.

[0067] It is known that semi-allogeneic T cells will be

rejected by the subject's immune system, but that rejection

requires about two weeks to develop. Hence, the semi-

allogeneic T cells will have the two-week window of

15 opportunity needed to exert neuroprotection. After two weeks,

the semi-allogeneic T cells will be rejected from the body of

the subject, but that rejection is advantageous to the subject

because it will rid the subject of the foreign T cells and

prevent any untoward consequences of the activated T cells.

20 The semi-allogeneic T cells thus provide an important safety

factor and are a preferred embodiment.

[0068] It is known that a relatively small number of HLA

class II molecules are shared by most individuals in a

population. For example, about 50% of the Jewish population

express the HLA-DR5 gene. Thus, a bank of specific T cells

reactive to NS-antigen epitopes that are restricted to HLA-DR5

would be useful in 50% of that population. The entire

population can be covered essentially by a small number of

5   additional T cell lines restricted to a few other prevalent

HLA molecules, such as DR1, DR4, DR2, etc. Thus, a functional

bank of uniform T cell lines can be prepared and stored for

immediate use in almost any individual in a given population.

Such a bank of T cells would overcome any technical problems

10   in obtaining a sufficient number of specific T cells from the

subject in need of neuroprotection during the open window of

treatment opportunity. The semi-allogeneic T cells will be

safely rejected after accomplishing their role of

neuroprotection. This aspect of the invention does not

15   contradict, and is in addition to the use of autologous T

cells as described herein.

[0069] The NS-specific activated T cells are preferably

non-attenuated, although attenuated NS-specific activated T

cells may be used. T cells may be attenuated using methods

20   well-known in the art including, but not limited to, by gamma-

irradiation, e.g.,, 1.5-10.0 Rads (Ben-Nun et al, 1981; Ben-

Nun and Cohen, 1982); and/or by pressure treatment, for

example as described in U.S. Patent No. 4,996,194 (Cohen et

al); and/or by chemical cross-linking with an agent such as

formaldehyde, glutaraldehyde and the like, for example as described in U.S Patent No. 4,996,194 (Cohen et al); and/or by cross-linking and photoactivation with light with a photoactivatable psoralen compound, for example as described in U.S. Patent No. 5,114,721 (Cohen et al); and/or by a cytoskeletal disrupting agent such as cytochalsin and colchicine, for example as described in U.S. Patent No. 4,996,194 (Cohen et al). In a preferred embodiment the NS-specific activated T cells are isolated as described below. T cells can be isolated and purified according to methods known in the art (Mor and Cohen, 1995). For an illustrative example, see Example 1, Materials and Methods.

[0070] Circulating T cells of a subject which recognize an NS-antigen are isolated and expanded using known procedures (Burns et al, 1983; Pette et al, 1990; Martin et al, 1990; Schluesener et al, 1985; Suruhan-Dires Keneli et al, 1993, which are incorporated herein by reference in their entirety). In order to obtain NS-specific activated T cells, T cells are isolated and the NS-specific activated T cells are then expanded.

[0071] The isolated T cells may be activated by exposure of the cells to one or more of a variety of natural or synthetic NS-specific antigens or epitopes as described in section on NS-specific antigens, analogs thereof, peptides derived

therefrom and analogs and derivatives thereof of said peptides

hereinafter. During *ex vivo* activation of the T cells, the T

cells may be activated by culturing in medium to which at

least one suitable growth promoting factor has been added,

5   such as cytokines, e.g., TNF-α, IL-2 and/or IL-4.

[0072] In one embodiment, the NS-specific activated T cells

endogenously produce a substance that ameliorates the effects

of injury or disease in the NS.

[0073] In another embodiment, the NS-specific activated T

10   cells endogenously produce a substance that stimulates other

cells, including, but not limited to, transforming growth

factor-β (TGF-β), nerve growth factor (NGF), neurotrophic

factor 3(NT-3), neurotrophic factor 4/5 (NT-4/5), brain

derived neurotrophic factor (BDNF); IFN-γ and IL-6, wherein the

15   other cells, directly or indirectly, ameliorate the effects of

injury or disease.

[0074] Following their proliferation *in vitro*, the T cells

are administered to a mammalian, preferably a human, subject.

T cell expansion is preferably performed using peptides

20   corresponding to sequences in a non-pathogenic, NS-specific,

self-protein.

[0075] A subject can initially be immunized with an NS-

specific antigen using a non-pathogenic peptide of the self-

protein. A T-cell preparation can be prepared from the blood

of such immunized subjects, preferably from T cells selected for their specificity towards the NS-specific antigen. The selected T cells can then be stimulated to produce a T cell line specific to the self-antigen (Ben-Nun and Cohen, 1982).

[0076] NS-specific antigen activated T cells, obtained as described above, can be used immediately or may be preserved for later use, e.g.,, by cryopreservation as described below. NS-specific activated T cells may also be obtained using previously cryopreserved T cells, i.e., after thawing the cells, the T cells may be incubated with NS-specific antigen, optimally together with thymocytes, to obtain a preparation of NS-specific activated T cells.

[0077] As will be evident to those skilled in the art, the T cells can be preserved, e.g.,, by cryopreservation, either before or after culture.

[0078] Cryopreservation agents which can be used include, but are not limited to, dimethyl sulfoxide (DMSO) (Lovelock and Bishop, 1959; Ashwood-Smith, 1961), polyvinylpyrrolidone (Rinfret, 1960), glycerol, polyethylene glycol (Sloviter and Ravdin, 1962), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe et al, 1962), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender et al, 1960), amino acids (Phan The Tran and Bender, 1960), methanol, acetamide, glycerol monoacetate (Lovelock, 1954),

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inorganic salts (Phan The Tran and Bender, 1960 and 1961) and DMSO combined with hydroxyethyl starch and human serum albumin (Zaroulis and Leiderman, 1980).

[0079] A controlled cooling rate is critical. Different cryoprotective agents (Rapatz et al, 1968) and different cell types have different optimal cooling rates. See, e.g.,, Rowe and Rinfret, 1962; Rowe, 1966; Lewis et al, 1967; Mazur, 1970) for effects of cooling velocity on survival of cells and on their transplantation potential. The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, e.g.,, a programmable freezing device or a methanol bath procedure.

[0080] Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling. Programmable controlled-rate freezers such as Cryomed or Planar permit tuning of the freezing regimen to the desired cooling rate curve.

[0081] After thorough freezing, cells can be rapidly transferred to a long-term cryogenic storage vessel. In one embodiment, samples can be cryogenically stored in mechanical freezers, such as freezers that maintain a temperature of about -80°C or about -20°C. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196°C) or its vapor. Such storage is greatly facilitated by the

availability of highly efficient liquid nitrogen refrigerators, which resemble large Thermos containers with an extremely low vacuum and internal super insulation, such that heat leakage and nitrogen losses are kept to an absolute minimum.

[0082] Considerations and procedures for the manipulation, cryopreservation, and long term storage of T cells can be found, for example, in the following references, incorporated by reference herein: Gorin, 1986; Bone-Marrow Conservation, Culture and Transplantation, 1968.

[0083] Other methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use, e.g.,, cold metal-mirror techniques. See Livesey and Linner, 1987; Linner et al, 1986; see also U.S. Patent No. 4,199,022 by Senken et al, U.S. Patent No. 3,753,357 by Schwartz, U.S. Patent No. 4,559,298 by Fahy.

[0084] Frozen cells are preferably thawed quickly (e.g.,, in a water bath maintained at 37-47°C) and chilled immediately upon thawing. It may be desirable to treat the cells in order to prevent cellular clumping upon thawing. To prevent clumping, various procedures can be used, including but not limited to the addition before or after freezing of DNAse (Spitzer et al, 1980), low molecular weight dextran and

citrate, citrate, hydroxyethyl starch (Stiff et al, 1983), or acid citrate dextrose (Zaroulis and Leiderman, 1980), etc.

[0085] The cryoprotective agent, if toxic in humans, should be removed prior to therapeutic use of the thawed T cells. One way in which to remove the cryoprotective agent is by dilution to an insignificant concentration.

[0086] Once frozen T cells have been thawed and recovered, they are used to promote neuroprotection as described herein with respect to non-frozen T cells. Once thawed, the T cells may be used immediately, assuming that they were activated prior to freezing. Preferably, however, the thawed cells are cultured before injection to the patient in order to eliminate non-viable cells. Furthermore, in the course of this culturing over a period of about one to three days, an appropriate activating agent can be added so as to activate the cells, if the frozen cells were resting T cells, or to help the cells achieve a higher rate of activation if they were activated prior to freezing. Usually, time is available to allow such a culturing step prior to administration as the T cells may be administered as long as a week after injury, and possibly longer, and still maintain their neuro-regenerative and neuroprotective effect.

[0087] To minimize secondary damage after nerve injury, patients can be treated by administering autologous or semi-

allogeneic T lymphocytes sensitized to at least one appropriate NS-antigen. As the window of opportunity has not yet been precisely defined, therapy should be administered as soon as possible after the primary injury to maximize the chances of success, preferably within about one week.

[0088] To bridge the gap between the time required for activation and the time needed for treatment, a bank with autologous, semi-allogeneic or allogeneic T cells can be established for future use.

[0089] Thus, in another embodiment, the invention provides cell banks that can be established to store NS-sensitized T cells for neuroprotective treatment of individuals at a later time, as needed.

[0090] In one embodiment, autologous T cells may be obtained from an individual and the cell bank will contain personal vaults of autologous T lymphocytes prepared for future use for neuroprotective therapy against secondary degeneration in case of NS injury. T lymphocytes are isolated from the blood, sensitized to a NS-antigen, and the cells are then frozen and suitably stored under the person's name, identity number, and blood group, in a cell bank until needed.

[0091] Additionally, autologous stem cells of the CNS can be processed and stored for potential use by an individual patient in the event of traumatic disorders of the NS such as

ischemia or mechanical injury, as well as for treating
neurodegenerative conditions such as Alzheimer's disease or
Parkinson's disease.

[0092] Alternatively, allogeneic or semi-allogeneic T cells
may be stored such that a bank of T cells of each of the most
common MHC-class II types are present. The semi-allogeneic or
allogeneic T cells are stored frozen for use by any individual
who shares one MHC type II molecule with the source of the T
cells.

[0093] In case an individual is to be treated for an
injury, preferably autologous stored T cells are used, but, if
autologous T cells are not available, then cells should be
used which share an MHC type II molecule with the patient, and
these would be expected to be operable in that individual.

[0094] The cells are preferably stored in an activated
state after exposure to an NS-antigen or peptide derived
therefrom. However, the cells may also be stored in a resting
state and activated once they are thawed and prepared for use.
The cell lines of the bank are preferably cryopreserved. The
cell lines are prepared in any way which is well known in the
art. Once the cells are thawed, they are preferably cultured
prior to injection in order to eliminate non-viable cells.
During this culturing, the cells can be activated or
reactivated using the same NS-antigen or peptide as used in

the original activation. Alternatively, activation may be achieved by culturing in the presence of a mitogen, such as phytohemagglutinin (PHA) or concanavalin A (preferably the former). This will place the cells into an even higher state of activation. The few days that it takes to culture the cells should not be detrimental to the patient as the treatment in accordance with the present invention may occuo still be effective. Alternatively, if time is of the essencer any time up to a week or more after the injury in order t, the stored cells may be administered immediately after thawing.

NS-SPECIFIC ANTIGENS, ANALOGS THEREOF, PEPTIDES DERIVED THEREFROM, AND ANALOGS AND DERIVATIVES THEREOF

[0095] The term "NS-specific antigen" as used herein refers to an antigen of the NS that specifically activates T cells such that following activation the activated T cells accumulate at a site of injury or disease in the NS of the patient.

[0096] The NS-specific antigen used according to the present invention may be an antigen obtained from NS tissue, preferably from tissue at a site of CNS injury or disease. It may be a crude NS-tissue preparation, e.g.,, derived from NS tissue obtained from mammalian NS that may include cells, both living or dead cells, membrane fractions of such cells or tissue, etc., and may be obtained by an NS biopsy or necropsy

from a mammal, preferably human, tissue including, but not limited to, from a site of CNS injury; from cadavers; and from cell lines grown in culture.

[0097] In one embodiment, the NS-specific antigen is an isolated or purified antigen. The NS-specific antigen may be isolated and purified by standard methods including chromatography (e.g.,, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of antigens. The functional properties may be evaluated using any suitable assay. Additionally, an NS-specific antigen may be a protein obtained by genetic engineering, chemically synthesized, etc.

[0098] In the practice of the invention, natural or synthetic NS-specific antigens are preferred and include, without being limited to, myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG), S-100, β-amyloid, Thy-1, P0, P2, neurotransmitter receptors, Nogo and Nogo receptor (NgR).

[0099] Specific illustrative examples of useful NS-specific antigens include but are not limited to, human MBP, depicted in Fig. 21 (SEQ ID NO:12); human PLP, depicted in Fig. 22 (SEQ ID NO:13); human MOG, depicted in Fig. 23 (SEQ ID NO:14), rat

Nogo A, B and C (Chen et al, 2000; WO 00/31235) (SEQ ID

NOs:18, 20 and 21), peptide p472 (SEQ ID NO:19), human Nogo A,

B and C (Prinjha et al, 2000) (SEQ ID NOs:23-25), and human or

mouse Nogo receptor (NgR) (Fournier et al, 2001) (SEQ ID

5   NOs:26 and 27, respectively).

[0100] Also encompassed by the present invention are

analogs of NS-specific antigens including, but not being

limited to, those molecules comprising regions that are

substantially homologous to the full-length NS-specific

10   antigen, or fragments thereof. In various embodiments, these

analogs will have at least 60% or 70% or 80% or 90% or 95%

identity over an amino acid sequence of identical size or when

compared to an aligned sequence in which the alignment is done

by a computer homology program known in the art or whose

15   encoding nucleic acid is capable of hybridizing to a coding

nucleotide sequence of the full-length NS-specific antigen,

under high stringency, moderate stringency, or low stringency

conditions. Computer programs for determining homology may

include, but are not limited to, TBLASTN, BLASTP, FASTA,

20   TFASTA, and CLUSTALW (Pearson and Lipman, 1988; Altschul et

al, 1990; Thompson, et al, 1994; Higgins, et al, 1996).

[0101] The NS-specific antigen analogs of the invention can

be produced by various methods known in the art. The

manipulations which result in their production can occur at

the gene or protein level. For example, a cloned gene

sequence can be modified by any of numerous strategies known

in the art (Maniatis, 1990). The sequence can be cleaved at

appropriate sites with restriction endonuclease(s), followed

5   by further enzymatic modification if desired, isolated, and

ligated *in vitro*.

[0102] Additionally, the coding nucleic acid sequence can

be mutated *in vitro* or *in vivo*, to create and/or destroy

translation, initiation, and/or termination sequences, or to

10 create variations in coding regions and/or form new

restriction endonuclease sites or destroy preexisting ones, to

facilitate further *in vitro* modification. Any technique for

mutagenesis known in the art can be used, including but not

limited to, chemical mutagenesis, *in vitro* site-directed

15 mutagenesis (Hutchinson, et al, 1978), etc.

[0103] Manipulations may also be made at the protein level.

Included within the scope of the invention are NS-specific

antigen derivatives which are differentially modified during

or after translation, e.g.,,, by glycosylation, acetylation,

20 phosphorylation, amidation, derivatization by known

protecting/blocking groups, proteolytic cleavage, linkage to

an antibody molecule or other cellular ligand, etc. Any of

numerous chemical modifications may be carried out by known

techniques, including but not limited to specific chemical

cleavage by cyanogen bromide, trypsin, chymotrypsin, papain,

V8 protease, $NaBH_4$; acetylation, formylation, oxidation,

reduction; metabolic synthesis in the presence of tunicamycin;

etc.

5   [0104] In a preferred embodiment, the invention relates to

peptides derived from NS-specific antigens or from analogs

thereof and to analogs or derivatives of said peptides, which

are functionally active, i.e., they are capable of displaying

one or more known functional activities associated with a

10   full-length NS-specific antigen. Such functional activities

include, but are not limited to, antigenicity (ability to

bind, or compete with an NS-antigen for binding, to an anti-

NS-specific antibody), immunogenicity (ability to generate

antibody which binds to an NS-specific protein), and ability

15   to interact with T cells, resulting in activation comparable

to that obtained using the corresponding full-length NS-

specific antigen. The crucial test is that the antigen which

is used for activating the T cells causes the T cells to be

capable of recognizing an antigen in the NS of the mammal

20   (patient) being treated.

[0105] The NS-antigen derived peptide may be either: (1)

an immunogenic peptide, i.e., a peptide that can elicit a

human T-cell response detected by a T-cell proliferation assay

or by cytokine, e.g., IFN-γ, IL-2, IL-4 or IL-10, production,

or (2) a "cryptic epitope" (also designated herein as "immunosilent" or "non-immunodominant" epitope), i.e., a peptide that by itself can induce a T-cell immune response that is not induced by the whole antigen protein (see Moalem et al, 1999).

[0106] A peptide derived from a NS-specific antigen preferably has a sequence comprised within the NS-specific antigen sequence and has at least 10, 13, 15, 18, 20 or 50 contiguous amino acids of the NS-specific antigen sequence. In one embodiment, the peptide derived from an NS-specific antigen is a "cryptic epitope" of the antigen. A cryptic epitope activates specific T cells after an animal is immunized with the particular peptide, but not with the whole antigen. Cryptic epitopes for use in the present invention include, but are not limited to, peptides of the MBP sequence: peptides p11-30, p51-70, p87-99, p91-110, p131-150, and p151-170. Such cryptic epitopes are particularly preferred as T cells activated thereby will accumulate at the injury site, but are particularly weak in autoimmunity. Thus, they would be expected to have fewer side effects.

[0107] In another embodiment, the peptide derived from an NS-specific antigen is an immunogenic epitope of the antigen.

[0108] Examples of further peptides according to the invention are immunogenic peptides derived from the Nogo

protein sequence such as, but not being limited to, the 18-mer

p472 Nogo peptide (SEQ ID NO:18) and peptides derived from the

Nogo receptor (Fournier et al, 2001) such as the 15-mer

peptides of the sequences:

5   S G V P S N L P Q R L A G R D (SEQ ID NO:28)

              T R S H C R L G Q A G S G S S (SEQ ID NO:29)

[0109] In still another embodiment of the invention, the

peptide is an analog of a peptide derived from an NS-specific

antigen that is immunogenic but not encephalitogenic. The

10 most suitable peptides for this purpose are those in which an

encephalitogenic self-peptide is modified at the T-cell

receptor (TCR) binding site and not at the MHC binding

site(s), so that the immune response is activated but not

anergized (Karin et al, 1998; Vergelli et al, 1996).

15 [0110] These analogs, also referred herein as modified

peptides or altered peptides, may be produced by replacement

of one or more amino acid residues of the peptide by other

amino acid residues, preferably in their TCR binding site.

Suitable replacements are those in which charged amino

20 residues like lysine, proline or arginine are replaced by

glycine or alanine residues. For example, altered peptides

can be produced from peptides p11-30, p51-70, p87-99, p91-110,

p131-150, and p151-170 of human MBP, for example from the p87-

99 peptide in which the lysine 91 is replaced by glycine

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and/or the proline 96 is replaced by an alanine residue, thus converting an encephalitogenic peptide in immunogenic but non-encephalitogenic peptide that still recognizes the TCR. In the same way, altered peptides can be produced from the

5   encephalitogenic p472 Nogo peptide (Nogo p623-640) by replacement of the lys 628 residue and from the Nogo receptor peptides above by replacement of the arg (R) residue by Val or Ala or another similar residue.

[0111] In addition, the analogs also comprise replacement

10   of one or more amino acid residues of the peptide or addition to the peptide of non-natural amino acids including, but not limited to, the D-isomers of the common amino acids, α-aminoisobutyric acid; 4-aminobutyric acid (Abu); 2-Abu (γ-Abu); 6-amino hexanoic acid (ε-Ahx); 2-aminoisobutyric acid (Aib);

15   3-aminopropionic acid; ornithine; norleucine (Nle); norvaline (Nva); hydroxyproline; sarcosine; citrulline; cysteic acid; t-butylglycine; t-butylalanine; phenylgylcine; cyclohexylalanine; β-alanine; fluoro-amino acids; designer amino acids such as β-methyl amino acids, Cα-methyl amino

20   acids, Nα-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0112] Furthermore, the invention also comprises chemical derivatives of the peptides of the invention including, but

not being limited to, esters of both carboxylic and hydroxy groups, amides, and the like.

[0113] The NS-specific antigen peptides of the invention can be chemically synthesized. For example, a peptide corresponding to a portion of an antigen which comprises the desired domain or which mediates the desired activity can be synthesized by use of a peptide synthesizer.

[0114] The functional activity of NS-specific antigens and peptides derived therefrom and analogs and derivatives thereof can be assayed by various methods known in the art, including, but not limited to, T-cell proliferation assays (Mor and Cohen, 1995) and cytokine production assays.

[0115] An NS-specific antigen or peptide derived therefrom or derivative thereof may be kept in solution or may be provided in a dry form, e.g., as a powder or lyophilizate, to be mixed with appropriate solution prior to use. They may be used both as ingredients of pharmaceutical compositions for neuroprotection and preventing or inhibiting the effects of injury or disease that result in NS degeneration or for promoting nerve regeneration in the NS, particularly in the CNS as well as for *in vivo* or *in vitro* activation of T cells.

NUCLEOTIDE SEQUENCES ENCODING NS-ANTIGENS AND PEPTIDES DERIVED THEREFROM

[0116] The present invention further provides pharmaceutical compositions comprising a therapeutically effective amount of a nucleotide sequence encoding an NS-specific antigen or a peptide derived therefrom or an analog thereof and methods of use of such compositions to promote nerve regeneration or for neuroprotection and prevention or inhibition of neuronal degeneration in the CNS or PNS in which the amount is effective to ameliorate the effects of an injury or disease of the NS.

[0117] Specific illustrative examples of useful nucleotide sequences encoding NS-specific antigens or peptides derived from an NS-specific antigen include, but are not limited to, nucleotide sequences encoding rat MBP, depicted in Fig. 15 (SEQ ID NO:1); human MBP, depicted in Fig. 16 (SEQ ID NO:2); human PLP, depicted in Figs. 17(A-F) (SEQ ID NOs:3-8); human MOG, depicted in Fig. 18 (SEQ ID NO:9); rat PLP and variant, depicted in Fig. 19 (SEQ ID NO:10); rat MAG, depicted in Fig. 20 (SEQ ID NO:11); rat Nogo (SEQ ID NO:17); and human Nogo (SEQ ID NO:22). Other illustrative examples are the nucleotide sequences disclosed in Chen et al (2000), Prinjha et al (2000) and Fournier et al (2001) (the contents of each of which being hereby incorporated herein by reference)

encoding rat and human Noga A, B and C and mouse and human
NgR.

THERAPEUTIC USES

[0118] The T cells, NS-specific antigens, analogs thereof,
peptides derived therefrom and analogs and derivatives
thereof, and nucleotide sequences described in the previous
sections and compositions comprising them may be used to
promote nerve regeneration or to confer neuroprotection and
prevent or inhibit secondary degeneration which may otherwise
follow primary NS injury, e.g.,, spinal cord injury, blunt
trauma, penetrating trauma, hemorrhagic stroke, ischemic
stroke or damages caused by surgery such as tumor excision.

[0119] In addition, such compositions may be used to
ameliorate the effects of disease that result in a
degenerative process, e.g.,, degeneration occurring in either
gray or white matter (or both) as a result of various diseases
or disorders, including, without limitation: diabetic
neuropathy, senile dementias, Alzheimer's disease, Parkinson's
disease, facial nerve (Bell's) palsy, glaucoma, Huntington's
chorea, amyotrophic lateral sclerosis (ALS), non-arteritic
optic neuropathy, intervertebral disc herniation, vitamin
deficiency, prion diseases such as Creutzfeldt-Jakob disease,
carpal tunnel syndrome, peripheral neuropathies associated
with various diseases, including but not limited to, uremia,

porphyria, hypoglycemia, Sjorgren Larsson syndrome, acute
sensory neuropathy, chronic ataxic neuropathy, biliary
cirrhosis, primary amyloidosis, obstructive lung diseases,
acromegaly, malabsorption syndromes, polycythemia vera, IgA-
5   and IgG gamma-pathies, complications of various drugs (e.g.,,
metronidazole) and toxins (e.g.,, alcohol or
organophosphates), Charcot-Marie-Tooth disease, ataxia
telangectasia, Friedreich's ataxia, amyloid polyneuropathies,
adrenomyeloneuropathy, Giant axonal neuropathy, Refsum's
10   disease, Fabry's disease, lipoproteinemia, etc.

[0120] In a preferred embodiment, the NS-specific activated
T cells, the NS-specific antigens, peptides derived therefrom,
analogs and derivatives thereof or the nucleotides encoding
said antigens, or peptides or any combination thereof of the
15   present invention are used to treat diseases or disorders
where promotion of nerve regeneration or prevention or
inhibition of secondary neural degeneration is indicated,
which are not autoimmune diseases or neoplasias. In a
preferred embodiment, the compositions of the present
20   invention are administered to a human subject.

[0121] While activated NS-specific T cells may have been
used in the prior art in the course of treatment to develop
tolerance to autoimmune antigens in the treatment of
autoimmune diseases, or in the course of immunotherapy in the

treatment of NS neoplasms, the present invention can also be used to ameliorate the degenerative process caused by autoimmune diseases or neoplasms as long as it is used in a manner not suggested by such prior art methods. Thus, for

5 example, T cells activated by an autoimmune antigen have been suggested for use to create tolerance to the autoimmune antigen and, thus, ameliorate the autoimmune disease. Such treatment, however, would not have suggested the use of T cells directed to other NS antigens or NS antigens which will

10 not induce tolerance to the autoimmune antigen or T cells which are administered in such a way as to avoid creation of tolerance. Similarly, for neoplasms, the effects of the present invention can be obtained without using immunotherapy processes suggested in the prior art by, for example, using an

15 NS antigen which does not appear in the neoplasm. T cells activated with such an antigen will still accumulate at the site of neural degeneration and facilitate inhibition of this degeneration, even though it will not serve as immunotherapy for the tumor *per se*.

20 [0122] Nogo protein or a fragment thereof which are active in inhibiting cell proliferation have been disclosed as useful for treatment of a neoplastic disease of the CNS such as glioma, glioblastoma, medulloblastoma, craniopharyngioma, ependyoma, neuroblastoma and retinoblastoma. The present

invention does not encompass the use of Nogo or a peptide derived therefrom for treatment of neoplasias in general, and for treatment of a neoplastic disease of the CNS, in particular.

5   **FORMULATIONS AND ADMINISTRATION**

[0123] The present invention also provides pharmaceutical compositions useful in methods to promote nerve regeneration or to confer neuroprotection and prevent or inhibit neuronal degeneration in the CNS or PNS, comprising a therapeutically

10   effective amount of at least one ingredient selected from the group consisting of:

[0124] (a) NS-specific activated T cells;

[0125] (b) a NS-specific antigen or an analog thereof;

[0126] (c) a peptide derived from an NS-specific antigen

15   or from an analog thereof, or an analog or derivative of said peptide;

[0127] (d) a nucleotide sequence encoding an NS-specific antigen or an analog thereof;

[0128] (e) a nucleotide sequence encoding a peptide

20   derived from an NS-specific antigen or from an analog thereof, or an analog of said peptide; or

[0129] (f) any combination of (a)-(e).

[0130] The compositions comprising ingredients (b) and/or (c) above are also effective to activate T cells *in vitro*,

wherein the activated T cells inhibit or ameliorate the effects of an injury or disease of the NS.

[0131] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[0132] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatin, starch, lactose or lactose monochydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

[0133] Methods of administration include, but are not limited to, parenteral, e.g.,, intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g.,, oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal, topical

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and intradermal routes. Administration can be systemic or local.

[0134] For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g.,, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g.,, lecithin or acacia); non-aqueous vehicles (e.g.,, almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g.,, methyl or propyl-p-hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g.,, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (e.g.,, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g.,, magnesium stearate, talc or silica); disintegrants (e.g.,, potato starch or sodium starch glycolate); or wetting agents (e.g.,, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art.

[0135] Preparations for oral administration may be also suitably formulated to give controlled release of the active compound.

[0136] The compositions may be formulated for parenteral administration by injection, e.g.,, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g.,, in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g.,, sterile pyrogen-free water, before use.

[0137] The compositions may also be formulated as rectal compositions such as suppositories or retention enemas, e.g.,, containing conventional suppository bases such as cocoa butter or other glycerides.

[0138] For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g.,, dichlorodifluoromethane, trichlorofluoro-methane, dichlorotetrafluoroethane, carbon dioxide or other

suitable gas. In the case of a pressurized aerosol the dosage
unit may be determined by providing a valve to deliver a
metered amount. Capsules and cartridges of, e.g.,, gelatin,
for use in an inhaler or insufflator may be formulated
5   containing a powder mix of the compound and a suitable powder
base such as lactose or starch.

[0139] In one embodiment, compositions comprising NS-
specific activated T cells, an NS-specific antigen or peptide
derived therefrom, or derivative thereof, or a nucleotide
10   sequence encoding such antigen or peptide, are formulated in
accordance with routine procedures as pharmaceutical
compositions adapted for intravenous or intraperitoneal
administration to human beings. Typically, compositions for
intravenous administration are solutions in sterile isotonic
15   aqueous buffer. Where necessary, the composition may also
include a solubilizing agent and a local anesthetic such as
lignocaine to ease pain at the site of the injection.
Generally, the ingredients are supplied either separately or
mixed together. Where the composition is to be administered
20   by infusion, it can be dispensed with an infusion bottle
containing sterile pharmaceutical grade water or saline.
Where the composition is administered by injection, an ampoule
of sterile water or saline for injection can be provided so
that the ingredients may be mixed prior to administration.

[0140] Pharmaceutical compositions comprising NS-specific antigen or peptide derived therefrom or derivative thereof may optionally be administered with an adjuvant.

[0141] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention.

[0142] In a preferred embodiment, the pharmaceutical compositions of the invention are administered to a mammal, preferably a human, shortly after injury or detection of a degenerative lesion in the NS. The therapeutic methods of the invention may comprise administration of an NS-specific activated T cell or an NS-specific antigen or peptide derived therefrom or derivative thereof, or a nucleotide sequence encoding such antigen or peptide, or any combination thereof. When using combination therapy, the NS-specific antigen may be administered before, concurrently or after administration of NS-specific activated T cells, a peptide derived from an NS-specific antigen or derivative thereof or a nucleotide sequence encoding such antigen or peptide.

[0143] In one embodiment, the compositions of the invention are administered in combination with one or more of the following: (a) mononuclear phagocytes, preferably cultured monocytes (as described in PCT publication No. WO 97/09985,

which is incorporated herein by reference in its entirety), that have been stimulated to enhance their capacity to promote neuronal regeneration; (b) a neurotrophic factor such as acidic fibroblast growth factor; and (c) an anti-inflammatory

5   therapeutic substance, e.g., an anti-inflammatory steroid, such as dexamethasone or methyl-prednisolone, or a non-steroidal anti-inflammatory peptide, such as Thr-Lys-Pro (TKP)).

[0144] In another embodiment, mononuclear phagocyte cells

10   according to PCT Publication No. WO 97/09985 and U.S. patent application Serial No. 09/041,280, filed March 11, 1998, are injected into the site of injury or lesion within the CNS, either concurrently, prior to, or following parenteral administration of NS-specific activated T cells, an NS-

15   specific antigen or peptide derived therefrom or derivative thereof, or a nucleotide sequence encoding such antigen or peptide

[0145] In another embodiment, administration of NS-specific activated T cells, NS-specific antigen or peptide sequence

20   encoding such antigen or peptide, may be administered as a single dose or may be repeated, preferably at 2-week intervals and then at successively longer intervals once a month, once a quarter, once every six months, etc. The course of treatment may last several months, several years or occasionally also

through the life-time of the individual, depending on the condition or disease which is being treated. In the case of a CNS injury, the treatment may range between several days to months or even years, until the condition has stabilized and there is no or only a limited risk of development of secondary degeneration. In chronic human disease or Parkinson's disease, the therapeutic treatment in accordance with the invention may be for life.

[0146] As will be evident to those skilled in the art, the therapeutic effect depends at times on the condition or disease to be treated, on the individual's age and health condition, on other physical parameters (e.g., gender, weight, etc.) of the individual, as well as on various other factors, e.g.,, whether the individual is taking other drugs, etc.

[0147] The optimal dose of the therapeutic compositions comprising NS-specific activated T cells of the invention is proportional to the number of nerve fibers affected by NS injury or disease at the site being treated. In a preferred embodiment, the dose ranges from about 5×10^6 to about 10^7 for treating a lesion affecting about 10^5 nerve fibers, such as a complete transection of a rat optic nerve, and ranges from about 10^7 to about 10^8 for treating a lesion affecting about $10^6 - 10^7$ nerve fibers, such as a complete transection of a human optic nerve. As will be evident to those skilled in the

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art, the dose of T cells can be scaled up or down in proportion to the number of nerve fibers thought to be affected at the lesion or site of injury being treated.

[0148] The following examples illustrate certain features of the present invention but are not intended to limit the scope of the present invention.

EXAMPLE 1:   ACCUMULATION OF ACTIVATED T CELLS IN INJURED OPTIC NERVE

MATERIALS AND METHODS

Animals

[0149] Female Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science (Rehovot, Israel), matched for age (8-12 weeks) and housed four to a cage in a light and temperature-controlled room.

Media

[0150] The T-cell proliferation medium contained the following: Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Israel) supplemented with 2mM L-glutamine (L-Glu, Sigma, USA), 5×10^{-5} M 2-mercaptoethanol (2-ME, Sigma), penicillin (100 IU/ml; Biological Industries), streptomycin (100 μ/ml; Biological Industries), sodium pyruvate (1 mM; Biological Industries), non-essential amino acids (1 ml/100 ml; Biological Industries) and autologous rat serum 1% (vol/vol) (Mor et al, 1990). Propagation medium contained: DMEM, 2-ME, L-Glu, sodium pyruvate, non-essential

amino acids and antibiotics in the same concentration as above

with the addition of 10% fetal calf serum (FCS), and 10% T

cell growth factor (TCGF) obtained from the supernatant of

concanavalin A-stimulated spleen cells (Mor et al, 1990).

5 **Antigens**

[0151] MBP from the spinal cords of guinea pigs was

prepared as described (Hirshfeld, et al, 1970). OVA was

purchased from Sigma (St. Louis, MO). The p51-70 of the rat

18.5kDa isoform of MBP (sequence: APKRGSGKDSHTRTTHYG) (SEQ ID

10 NO:15) and the p277 peptide of the human hsp60 (sequence:

VLGGGCALLRCPALDSLTPANED) (SEQ ID NO:16) (Elias et al, 1991)

were synthesized using the 9-fluorenylmethoxycarbonyl (Fmoc)

technique with an automatic multiple peptide synthesizer (AMS

422, ABIMED, Langenfeld, Germany). The purity of the peptides

15 was analyzed by HPLC and amino acid composition.

T Cell Lines

[0152] T-cell lines were generated from draining lymph node

cells obtained from Lewis rats immunized with an antigen

(described above in Antigens). The antigen was dissolved in

20 PBS (1 mg/ml) and emulsified with an equal volume of IFA

(Difco Laboratories, Detroit, Michigan) supplemented with 4

mg/ml *Mycobacterium tuberculosis* (Difco 15 Laboratories,

Detroit, MI). The emulsion (0.1 ml) was injected into hind

foot pads of the rats. Ten days after the antigen was

injected, the rats were killed and draining lymph nodes were surgically removed and dissociated. The cells were washed and activated with the antigen (10 µg/ml) in proliferation medium (described above in Media). After incubation for 72 h at 37°C,

5　90% relative humidity and 7% CO_2, the cells were transferred to propagation medium (described above in Media). Cells were grown in propagation medium for 4-10 days before being re-exposed to antigen (10 µg/ml) in the presence of irradiated (2000 rad) thymus cells (10^7 cells/ml) in proliferation medium.

10　The T cell lines were expanded by repeated re-exposure and propagation.

Crush Injury of Rat Optic Nerve

[0153] Crush injury of the optic nerve was performed as previously described (Duvdevani et al, 1990). Briefly, rats

15　were deeply anesthetized by i.p. injection of Rompum (xylazine, 10 mg/kg; Vitamed, Israel) and Vetaler (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, Iowa). Using a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised

20　lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. Using calibrated cross-action forceps, a moderate crush injury was inflicted on the optic nerve, 2 mm

form the eye (Duvdevani et al, 1990). The contralateral nerve was left undisturbed and was used as a control.

Immunocytochemistry of T Cells

[0154] Longitudinal cryostat nerve sections (20 µm thick) were picked up onto gelatin glass slides and frozen until preparation for fluorescent staining. Sections were thawed and fixed in ethanol for 10 minutes at room temperature, washed twice with double-distilled water (ddH$_2$O), and incubated for 3 minutes in PBS containing 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20; Sigma, USA). Sections were then incubated for 1 hr at room temperature with a mouse monoclonal antibody directed against rat T cell receptor (TCR) (1:100, Hunig et al, 1989), in PBS containing 3% FCS and 2% BSA. After three washes with PBS containing 0.05% Tween-20, the sections were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (with minimal cross-section to rat, human, bovine and horse serum proteins) (Jackson ImmunoResearcch, West Grove, PA) for one hour at room temperature. The sections were then washed with PBS containing Tween-20 and treated with glycerol containing 1,4-diazobicyclo-(2,2,2) octane (Sigma), to inhibit quenching of fluorescence. The sections were viewed with a Zeis microscope and cells were counted. Staining in the absence of first antibody was negative.

RESULTS

[0155] Fig. 1 shows accumulation of T cells measured immunohistochemically. The number of T cells was considerably higher in injured nerves rats injected with anti-MBP, anti-OVA or anti-p277 cells; statistical analysis (one-way ANOVA) showed significant differences between T cell numbers in injured optic nerves of rats injected with anti-MBP, anti-OVA, or anti-p277 T cells and in injured optic nerves of rats injected with PBS ($P<0.001$); and between injured optic nerves and uninjured optic nerves of rats injected with anti-MBP, anti-OVA, or anti-p277 T cells ($P<0.001$).

EXAMPLE 2: NEUROPROTECTION BY AUTOIMMUNE ANTI-MBP T CELLS
MATERIAL AND METHODS

[0156] Animals, media, antigens, crush injury of rat optic nerve, sectioning of nerves, T cell lines, and immunolabeling of nerve sections are described in Example 1, *supra*.

Retrograde Labeling and Measurement of Primary Damage and Secondary Degeneration

[0157] Primary damage of the optic nerve axons and their attached RGCs were measured after the immediate post-injury application of the fluorescent lipophilic dye 4-Di-10-Asp) (Molecular Probes Europe BV, Netherlands) distal to the site of injury. Only axons that are intact are capable of transporting the dye back to their cell bodies; therefore, the number of labeled cell bodies is a measure of the number of

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axons that survived the primary damage. Secondary

degeneration was also measured by application of the dye

distal to the injury site, but two weeks after the primary

lesion was inflicted. Application of the neurotracer dye

5   distal to the site of the primary crush after two weeks

ensures that only axons that survived both the primary damage

and the secondary degeneration will be counted. This

approach makes it possible to differentiate between neurons

that are still functionally intact and neurons in which the

10   axons are injured but the cell bodies are still viable, as

only those neurons whose fibers are morphologically intact can

take up dye applied distally to the site of injury and

transport it to their cell bodies. Using this method, the

number of labeled RGCs reliably reflects the number of still-

15   functioning neurons. Labeling and measurement were done by

exposing the right optic nerve for a second time, again

without damaging the retinal blood supply. Complete axotomy

was done 1-2 mm from the distal border of the injury site and

solid crystals (0.2-0.4 mm in diameter) of 4-Di-10-Asp were

20   deposited at the site of the newly formed axotomy. Uninjured

optic nerves were similarly labeled at approximately the same

distance from the globe. Five days after dye application, the

rats were killed. The retina was detached from the eye,

prepared as a flattened whole mount in 4% paraformaldehyde

solution and examined for labeled RGCs by fluorescence microscopy. The percentage of RGCs surviving secondary degeneration was calculated using the following formula: (Number of spared neurons after secondary degeneration)/

5 (Number of spared neurons after primary damage) x 100.

Electrophysiological Recordings

[0158] Nerves were excised and their compound action potentials (CAPs) were recorded *in vitro* using a suction electrode experimental set-up (Yoles et al, 1996). At

10 different times after injury and injection of T cells or PBS, rats were killed by intraperitoneal injection of pentobarbitone (170 mg/kg) (CTS Chemical Industries, Israel). Both optic nerves were removed while still attached to the optic chiasma, and were immediately transferred to a vial

15 containing a fresh salt solution consisting of 126 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_2 26 mM $NaHCO_3$ 2 mM $MgSO_4$, 2 mM $CaCl_2$ and 10 mM D-glucose, aerated with 95% O_2 and 5% CO_2 at room temperature. After 1 hour, electrophysiological recordings were made. In the injured nerve, recordings were made in a

20 segment distal to the injury site. This segment contains axons of viable RGCs that have escaped both primary and secondary damage, as well as the distal stumps of non-viable RGCs that have not yet undergone Wallerian degeneration. The nerve ends were connected to two suction Ag-AgCl electrodes

immersed in the bathing solution at 37°C. A stimulating pulse was applied through the electrode, and the CAP was recorded by the distal electrode. A stimulator (SD9; Grass Medical Instruments, Quincy, Massachusetts) was used for supramaximal

5 electrical stimulation at a rate of 1 pps to ensure stimulation of all propagating axons in the nerve. The measured signal was transmitted to a microelectrode AC amplifier (model 1800; A-M Systems, Everett, WA). The data were processed using the LabView 2.1.1 data acquisition and

10 management system (National Instruments, Austin, Texas). For each nerve, the difference between the peak amplitude and the mean plateau of eight CAPs was computed and was considered as proportional to the number of propagating axons in the optic nerve. The experiments were done by experimenters "blinded",

15 to sample identity. In each experiment the data were normalized relative to the mean CAP of the uninjured nerves from PBS-injected rats.

Clinical Evaluation of Experimental Autoimmune Encephalomyelitis (EAE)

20 [0159] Clinical disease was scored every 1 to 2 days according to the following neurological scale: 0, no abnormality; 1, tail atony; 2, hind limb paralysis; 3, paralysis extending to thoracic spine; 4, front limb paralysis; 5, moribund state.

Neuroprotection BY Autoimmune Anti-MBP T Cells

[0160] Morphological analyses were done to assess the
effect of the T cells on the response of the nerve to injury,
and specifically on secondary degeneration. Rats were
injected intraperitoneally immediately after optic nerve
injury with PBS or with $1x10^7$ activated T cells of the various
cell lines. The degree of primary damage to the optic nerve
axons and their attached RGCs was measured by injecting the
dye 4-Di-10-Asp distal to the site of the lesion immediately
after the injury. A time lapse of 2 weeks between a moderate
crush injury and dye application is optimal for demonstrating
the number of still viable labeled neurons as a measure of
secondary degeneration, and as the response of secondary
degeneration to treatment. Therefore, secondary degeneration
was quantified by injecting the dye immediately or 2 weeks
after the primary injury, and calculating the additional loss
of RGCs between the first and the second injections of the
dye. The percentage of RGCs that had survived secondary
degeneration was then calculated. The percentage of labeled
RGCs (reflecting still-viable neurons) was significantly
greater in the retinas of the rats injected with anti-MBP T
cells than in the retinas of the PBS-injected control rats
(Fig. 2). In contrast, the percentage of labeled 30 RGCs in

the retinas of the rats injected with anti-OVA or anti-p277 T cells was not significantly greater than that in the control retinas. Thus, although the three T cell lines accumulated at the site of injury, only the MBP-specific autoimmune T cells had a substantial effect in limiting the extend of secondary degeneration. Labeled RGCs of injured optic nerves of rats injected with PBS (Fig. 3A), with anti-p277 T cells (Fig. 3B) or with anti-MBP T cells (Fig. 3C) were compared morphologically using micrographs.

Clinical Severity Of EAE

[0161] Animals were injected i.p. with 10^7 T_{MBP} cells with or without concurrent optic nerve crush injury. The clinical course of the rats injected with the T_{MBP} cells was evaluated according to the neurological paralysis scale. Each group contained 5-9 rats. The functional autoimmunity of the injected anti-MBP T cells was demonstrated by the development of transient EAE in the recipients of these cells. As can be seen in Fig. 4A, the course and severity of the EAE was not affected by the presence of the optic nerve crush injury.

Survival of RGCS in Non-Injured Nerves

[0162] Animals were injected i.p. with 10^7 T_{MBP} cells or PBS. Two weeks later, 4-Di-10-Asp was applied to the optic nerves. After five days the retinas were excised and flat mounted. Labeled RGCs from five fields (located at approximately the

same distance from the optic disk), in each retina were counted and their average number per are (mm^2) was calculated.

[0163] As can be seen in Fig. 4B, there is no difference in the number of surviving RGCs per area (mm^2) in non-injured

5 optic nerves of rats injected with anti-MBP T cells compared to in rats injected with PBS.

Neuroprotection by T Cells Reactive to a Cryptic Epitope – (p51-70)MBP

[0164] To determine whether the neuroprotective effect of

10 the anti-MBP T cells is correlated with their virulence, the effect of T cells reactive to a "cryptic" epitope of MBP, the peptide 51-70 (p51-70) was examined. "Cryptic" epitopes activate specific T cells after an animal is immunized with the particular peptide, but not with the whole antigen (Mor et

15 al, 1995). The T cell line reactive to the whole MBP and the T cell line reactive to the cryptic epitope p51-70 were compared for the severity of the EAE they induced, and for their effects on secondary degeneration. In rats injected with the T cell line reactive to the cryptic epitope, disease

20 severity (as manifested by the maximal EAE score) was significantly lower than that in rats injected with the T cell line reactive to the whole protein (Table 1). Whereas anti-MBP T cells caused clinical paralysis of the limbs, rats injected with the anti-p51-70 T cells developed only tail

25 atony, not hind limb paralysis, and almost none showed

weakness of the hind limbs. Despite this difference in EAE

severity, the neuroprotective effect of the less virulent

(anti-p51-70) T cells was similar to that of the more virulent

(anti-MBP) T cells (Fig. 5). The percentage of RGCs surviving

5 secondary degeneration in the retinas of rats injected with

either of the lines was significantly higher than in the

retinas of the PBS-injected rats. Thus, there was no

correlation between the neuroprotective effect of the

autoimmune T cells and their virulence. It is possible that

10 the anti-p51-70 T cells encounter little antigen in the intact

CNS, and therefore cause only mild EAE. Their target antigen

may however become more available after injury, enabling these

T cells to exert a neuroprotective effect.

Table 1
Anti-MBP and Anti-p501-70 T Cells Vary in Pathogenicity

T Cell Line	Clinical EAE	Mean Max. Score
Whole MBP	Moderate to Severe	2.00 + 0.2
p51-70 of MBP	Mild	0.70 + 0.2

Immediately after optic nerve crush injury, Lewis rats were injected with activated anti-MBP T cells or anti-p51-70 T cells. The clinical course of EAE was evaluated according to the neurological paralysis scale. The mean maximal (max.) score ± s.e.m. was calculated as the average maximal score of all the diseased rats in each group. The table is a summary of nine experiments. Each group contains five to ten rats. Statistical analysis showed a significant difference between the mean maximal score of rats injected with anti-MBP T cells and that of rats injected with anti-p51-70 T cells ($P=0.039$, Student's t-test).

Electrophysiological Activity

[0165] To confirm the neuroprotective effect of the anti-MBP T cells, electrophysiological studies were done. Immediately after optic nerve injury, the rats were injected intraperitoneally with PBS or with 1×10^7 activated anti-MBP or anti-OVA T cells. The optic nerves were excised 7, 11 or 14 days later and the CAPs, a measure of nerve conduction, were recorded from the injured nerves. On day 14, the mean CAP amplitudes of the distal segments recorded from the injured nerves obtained from the PBS-injected control rats were 33% to 50% of those recorded from the rats injected with the anti-MBP T cells (Fig. 6A, Table 2). As the distal segment of the injured nerve contains both neurons that escaped the primary insult and injured neurons that have not yet degenerated, the observed neuroprotective effect could reflect the rescue of spared neurons, or a delay of Wallerian degeneration of the injured neurons (which normally occurs in the distal stump), or both. No effect of the injection of anti-MBP T cells on the mean CAP amplitudes of uninjured nerves was observed (Fig. 6B, Table 2). It is unlikely that the neuroprotective effect observed on day 14 could have been due to the regrowth of nerve fibers, as the time period was too short for this.

[0166] The strong neuroprotective effect of the anti-MBP T cells seen on day 14 was associated with a significantly decreased CAP amplitude recorded on day 7 (Table 2). The anti-MBP T cells manifested no substantial effect on the uninjured nerve on day 7, indicating that the reduction in electrophysiological activity observed in the injured nerve on day 7 might reflect the larger number of T cells present at the injury site relative to the uninjured nerve (Fig. 1). The observed reduction in CAP amplitude in the injured nerve on day 7 reflected a transient resting state in the injured nerve. This transient effect has not only disappeared, but was even reversed by day 14 (Table 2). Early signs of the neuroprotective effect could already be detected on day 11 in the rats injected with anti-OVA T cells, no reduction in CAP amplitude on day 7 could be detected in either the injured or the uninjured nerves, and no neuroprotective effect was observed on day 14 (Table 2). Thus, it seems that the early reduction in CAP and the late neuroprotection shown specifically by the anti-MBP T cells are related.

TABLE 2
Transient Reduction in Electrophysiological Activity of the Injured Optic Nerve Induced by Anti-MBP T Cells, Followed by a Neuroprotective Effect

	Uninjured Optic Nerve		Injured Optic Nerve	
	Day 7	Day 14	Day 7	Day 14
Ratio (&) T_{MPB}/PBS	89.9$\pm$9.4 (n=22)	101.2$\pm$22.7 (n=10)	63.8$^*\pm$14.9 (n=17)	243.1**$\pm$70.8 (n=8)
Ratio (%) T_{OVA}/PBS	109.7$\pm$13. (n=11)	92.5$\pm$12.6 (n=3)	125.5$\pm$24.4 (n=11)	107.3$\pm$38.9 (n=4)

Immediately after optic nerve injury, rats were injected with PBS or with activated anti-MBP or anti-OVA T cells. After 7 or 14 days, the CAPs of injured and uninjured nerves were recorded. Ratios were calculated for uninjured nerves as (mean CAP of uninjured nerves from T cell-injected rats/mean CAP of uninjured nerves from PBS-injected rats) x 100, or for injured nerves as (mean CAP of injured nerves from T cell-injected rats/mean CAP of injured nerves from PBS-injected rats) x 100. The *P* value was calculated by comparing the logarithms of the normalized CAP amplitudes of nerves from PBS-injected rats and rats injected with T cells, using the unpaired Student's test, $^*P<0.05$; $^{**}P<0.001$ n=sample size.

Neuroprotection in Spinal Cord Injury by Anti-MBP T Cells

Materials and Methods

[0167] Animals, antigens (MBP, OVA) and T cell lines were as described hereinbefore in Example 6 animals, antigens and T cell lines, respectively.

[0168] Contusion. Adult rats (300 to 350g) were anesthetized and the spinal cord was exposed by laminectomy at the level of T7-T8. One hour after induction of anesthesia, a 10-gram rod was dropped onto the laminectomized cord from a height of 50 mm. The impactor device (designed by Prof. Wise Young) allowed, for each animal, measurement of the trajectory

of the rod and its contact with the spinal cord to allow

uniform lesion. Within an hour of the contusion, rats were

injected i.p., on a random basis, with either 10^7 cells

(specific to either MBP or OVA, depending on the experimental

5   design) or with PBS. Bladder expression was done at least

twice a day (particularly during the first 48 h after injury,

when it was done 3 times a day) until the end of the second

week, by which time the rats had developed autonomous bladder

voidance. Approximately twice a week, locomotor activity (of

10   the trunk, tail and hind limbs) in an open field was evaluated

by placing the rat for 4 min in the middle of a circular

enclosure made of molded plastic with a smooth, non-slip floor

(90 cm diameter, 7 cm wall height).

Results

15   [0169] The present study of spinal cord neuroprotection was

prompted by the previous example that partial injury to an

optic nerve can be ameliorated administering T cells directed

to a CNS self-antigen. The question was whether autoimmune T

cells could have a beneficial effect on recovery from

20   traumatic spinal cord injury with its greater mass of injured

CNS tissue and the attendant spinal shock.

[0170] Adult Lewis rats were subjected to a calibrated

spinal cord contusion produced by dropping a 10-gram weight

from a height of 50 mm onto the laminectomized cord at the

level of T7-T8 (see Basso et al, 1996). The rats were then

injected intraperitoneally with autoimmune T cells specific to

MBP. Control rats were similarly injured but received either

no T cells or T cells specific to the non-self antigen

5   ovalbumin (OVA). Recovery of the rats was assessed every 3 to

4 days in terms of their behavior in an open-field locomotion

test, in which scores range from 0 (complete paraplegia) to 21

(normal mobility). The locomotor performance of the rats was

judged by observers blinded to the identity of the treatment

10 received by the rats. Included in the study was a group of

uninjured, sham-operated (laminectomized but not contused)

rats that were injected with anti-MBP T cells to verify the

activity of the T cells. In all the sham-operated rats, the

anti-MBP T cells induced clinical EAE, which developed by day

15 4, reached a peak at day 7 and resolved spontaneously by day

11. Note, therefore, that at the early post-traumatic stage,

any effect of the autoimmune T cells on the injured spinal

cord, whether positive or negative, would be transiently

masked both by spinal shock and by the paralysis of EAE.

20   [0171] Indeed, none of the rats with contused spinal cords

showed any locomotor activity in the first few days after the

contusion (Fig.7A). Interestingly, however, the rats treated

with anti-MBP T cells recovered earlier from spinal shock; on

day 11, for example, when no recovery could be detected in any

of the untreated control rats, significant improvement was
noted in the T cell-treated rats (Fig. 7A). At all time
points thereafter, the rats that had received the autoimmune T
cells showed better locomotor recovery than did the untreated

5   injured rats (Fig.7A). Thus the autoimmune T cells, in spite
of being encephalitogenic, did confer significant
neuroprotection. Moreover, the phase of neuroprotective
activity coincided with the phase of immune paralysis,
supporting our suggestion that neuroprotection might be

10   related to transient paralysis.

[0172] By one month after trauma the rats in both groups
had reached a maximal behavioral score, which then remained at
plateau for at least 3 months of follow-up. In the untreated
rats, maximal recovery of locomotor behavior, as noted in

15   previous reports of similarly severe contusion (Basso et al,
1996), was marked by some ineffectual movement of hind-limb
joints, but the rats showed no ability to support their body
weight and walk, and obtained a score of 7.3 ± 0.8 (mean ±
SEM). In contrast, the average score of the rats that had

20   been treated with the anti-MBP T cells was 10.2 ± 0.8, and in
some rats the value was as high as 13. All the rats in the
treated group could support their body weight and some could
frequently walk in a coordinated fashion. The difference
between the two groups, based on 2-factor repeated ANOVA, was

statistically significant (p<0.05). The recovery curve based on locomotor activity is nonlinear. The above-described increase in motor activity seen after treatment with the anti-MBP T cells could result from much higher percentage of spared tissue based on a linear regression curve on which the behavioral score is correlated with the amount of neural spinal cord tissue (for example, a difference between 11 and 7 on the locomotion score would be read as a difference between 30% and less than 10% of spared tissue).

[0173] In another set of experiments the rats were subjected to a more severe insult, resulting in a functional score of 1.9 ± 0.8 (mean $\pm$ SEM) in the untreated group and 7.7 ± 1.4 in the treated group (Fig. 7B). This difference of more than 3 fold in behavioral scores was manifested by the almost total lack of motor activity in the control rats as compared with the ability of the autoimmune T cell-treated rats to move all their joints. The beneficial effect was specific to treatment with anti-MBP T cells; no effect was observed after treatment with T cells specific to the non-self antigen OVA (data not shown). The positive effect of the autoimmune T cells seems to be expressed in the preservation of CNS tissue that escaped the initial lesion, i.e., in neuroprotection. Therefore, the magnitude of the effect would be inherently limited by the severity of the insult; the more severe the

lesion, the less the amount of spared tissue amenable to
neuroprotection.

[0174] To determine whether clinical recovery could be
explained in terms of preservation of spinal axons, we
performed retrograde labeling of the descending spinal tracts
by applying the dye rhodamine dextran amine (Brandt et al,
1992) at T12, below the site of damage. The number of dye-
stained cells that could be counted in the red nucleus of the
brain constituted a quantitative measure of the number of
intact axons traversing the area of contusion. Sections of
red nuclei from injured rats treated with anti-MBP T cells
(Fig. 8) contained 5-fold more labeled cells than sections
taken from the untreated injured rats. Photomicrographs of
red nuclei taken from rats treated with anti-MBP T cells (with
an open field score of 10) and from PBS-treated rats (with a
score of 6) are shown in Fig. 8. These findings indicate that
the reduction in injury-induced functional deficit observed in
the T cell-treated rats can be attributed to the sparing of
spinal tracts, resulting in a higher degree of neuron
viability.

[0175] After a follow-up of more than 3 months, when the
locomotor activity scores had reached a plateau, the site of
injury of three of PBS-treated animals and three animals
treated with anti-MBP T cells were analyzed by diffusion-

weighted MRI. The cords were excised in one piece from top to bottom and were immediately placed in fixative (4% paraformaldehyde). Axial sections along the excised contused cord were analyzed. Fig. 9 shows the diffusion anisotropy in axial sections along the contused cord of a rat treated with autoimmune T cells, as compared with that of PBS-treated control rat. The images show anisotropy in the white matter surrounding the gray matter in the center of the cord. Sections taken from the lesion sites of PBS-treated control rats show limited areas of anisotropy, which were significantly smaller than those seen at comparable sites in the cords of the rats treated with the anti-MBP T cells. Quantitative analysis of the anisotropy, reflecting the number of spared fibers, is shown in Fig. 9. The imaging results show unequivocally that, as a result of the treatment with the autoimmune anti-MBP T cells, some spinal cord tracts had escaped the degeneration that would otherwise have occurred.

Discussion of Results

[0176] No cure has yet been found for spinal cord lesions, one of the most common yet devastating traumatic injuries in industrial societies. It has been known for more that 40 years that CNS neurons, unlike neurons of the PNS, possess only a limited ability to regenerate after injury. During the last two decades, attempts to promote regeneration have

yielded approaches that lead to partial recovery. In the last few years it has become apparent that, although most of the traumatic injuries sustained by the human spinal cord are partial, the resulting functional loss is nevertheless far worse than could be accounted for by the severity of the initial insult; the self-propagating process of secondary degeneration appears to be decisive.

[0177] A substantial research effort has recently been directed to arresting injury-induced secondary degeneration. All attempts up to now have been pharmacologically based, and some have resulted in improved recovery from spinal shock. The present study, in contrast, describes a cell therapy that augments what seems to be a natural mechanism of self-maintenance and leads, after a single treatment, to long-lasting recovery. The extent of this recovery appears to exceed that reported using pharmacological methods.

[0178] In most tissues, injury-induced damage triggers a cellular immune response that acts to protect the tissue and preserve its homeostasis. This response has been attributed to macrophages and other cells comprising the innate arm of the immune system. Lymphocytes, which are responsible for adaptive immunity, have not been thought to participate in tissue maintenance. Adaptive immunity, according to traditional teaching, is directed against foreign dangers.

Our studies now show, however, that the adaptive T cell immune response can be protective even when there is no invasion by foreign pathogens. In the case of tissue maintenance, the specificity of the T cells is to tissue self-antigens.

5   [0179] Our observation of post-traumatic CNS maintenance by autoimmune T cells suggests that we might do well to reevaluate some basic concepts of autoimmunity. T cells that are specific to CNS self antigens in general, and to MBP in particular, have long been considered to be only detrimental to health. In the present study, however, the same T cell preparation that can produce EAE in the undamaged CNS was found to be neuroprotective in the damaged spinal cord, suggesting that the context of the tissue plays an important part in determining the outcome of its interaction with T cells. It would seem that the tissue deploys specific signals to elicit particular T cell behaviors. Among such signals are co-stimulatory molecules, particularly members of the B7 family (Lenchow et al, 1996). As shown hereinafter, the injured rat optic nerve transiently expresses elevated levels of the co-stimulatory molecule B7.2, which is constitutively expressed at low levels in the rat CNS white matter and which is thought to be associated with regulation of the cytokine profile of the responding T cells (Weiner, 1997). The early post-injury availability of the exogenous anti-MBP T cells,

coinciding with the observed early post-injury increase in B7.2, would support the idea that signals expressed by the tissue might modulate the T cell response. It is thus conceivable that anti-MBP T cells which cause a monophasic autoimmune disease upon interacting with a healthy CNS nerve, might implement a maintenance program when they interact with damaged CNS tissue expressing increased amounts of B7.2 and probably other co-stimulatory molecules. The neuroprotective effects of the T cells may be mediated, at least in part, by antigen-dependent regulation of specific cytokines or neurotrophic factors (Kerschensteiner et al, 1999) produced locally at the site of injury.

[0180] Thus, the present invention is also directed to manipulating B7.2 co-stimulatory molecule to prevent or inhibit neuronal degeneration and ameliorate the effects of injury to or disease of the nervous system. B7.2 molecule can be up-regulated for this purpose, using drugs or by genetic manipulation, without undue experimentation.

[0181] In a recent study, it was reported that injury to the spinal cord triggers a transient autoimmune response to MBP (Popovich et al, 1996). However, whether that response is detrimental or beneficial remained an open question (Popovich et al, 1997). From our present data, it would appear that the activation of anti-MBP T cells could indeed be beneficial.

However, a supplement of exogenous autoimmune T cells may be required to overcome the restrictions on immune reactivity imposed by the immune-privilege of the CNS (Streilein, 1995). The finding that autoimmune response can be advantageous suggests that natural autoimmune T cells may have undergone positive selection during ontogeny, as proposed by the theory of the immunological homunculus (Cohen, 1992), and are not merely a default resulting from the escape from negative selection of T cells that recognize self antigens (Janeway, 1992). Such a response could then be considered as a mechanism of potential physiological CNS self-maintenance, which is, however, not sufficient for the purpose because of the immune-privileged character of the CNS.

[0182] A single injection of autoimmune T cells lasted for at least 100 days. Thus, this procedure offers a form of self-maintenance. This specific autoimmune response, when properly controlled, is useful as part of a self-derived remedy for spinal cord injury.

EXAMPLE 3: NEUROPROTECTIVE EFFECTS OF A NS-SPECIFIC ANTIGEN PEPTIDE — MOG p35-55

MATERIALS AND METHODS

[0183] Animals, crush injury of rat optic nerve, and retrograde labeling are described above in Examples 3 and 4. A peptide based on amino acids 35-55 of MOG (MOG p35-55) was chemically synthesized at the Weizmann Institute, Israel.

Inhibition of Secondary Degeneration

[0184] Rats were injected intradermally in the footpads with MOG p35-55 (50 μg/animal) and IFA, or PBS, ten days prior to optic nerve crush injury. RGCs were assessed two weeks after injury using retrograde labeling as described above. The number of RGCs in rats injected with PBS or MOG p35-55 was expressed as a percentage of the total number of neurons in rats injected with MOG p35-55 in the absence of crush injury.

RESULTS

[0185] As shown in Fig. 10, the number of labeled RGCs (indicating viable axons) was about 12.5-fold greater in animals injected with MOG p35-55 compared to animals receiving PBS.

EXAMPLE 4: NEUROPROTECTIVE EFFECTS OF MBP ADMINISTERED ORALLY
MATERIALS AND METHODS

[0186] Animals, crush injury of rat optic nerve, and retrograde labeling of RGCs are described above in Examples 3 and 4.

Inhibition of Secondary Degeneration

[0187] Bovine MBP (Sigma, Israel) (1 mg/dose) was administered to rats by gavage using a blunt needle. MBP was administered 5 times, every third day, beginning 2 weeks prior to optic nerve crush injury. The number of RGCs in treated animals was expressed as a percentage of the total number of

neurons in animals subjected to optic nerve crush injury but which did not receive MBP.

RESULTS

[0188] As shown in Fig. 11, the number of labeled RGCs was about 1.3 fold greater in animals treated with MBP compared to untreated animals.

THE B7.2 CO-STIMULATORY MOLECULE IS ASSOCIATED WITH POST-TRAUMATIC MAINTENANCE OF THE OPTIC NERVE BY ORAL ADMINISTRATION OF MBP

Introduction

[0189] Autoimmune T cells can under certain conditions be beneficial to traumatized CNS axons. The effect of such T cells on the damaged tissue might be influenced by the nature and amount of the co-stimulatory molecules it expresses. We show that the B7.2 co-stimulatory molecule is constitutively expressed in the intact rat optic nerve, and after injury is up-regulated at the margins of the injury site. Pre-injury induction of oral tolerance to MBP resulted in a further post-injury increase in B7.2 at the margins and at the injury site itself, as well as a better preservation of the traumatized nerve. Thus, B7.2 expression in the brain and its up-regulated after trauma seem to be directly related to post-traumatic maintenance displayed by autoimmune T cells.

[0190] Neuronal injury in the CNS causes degeneration of directly damaged fibers as well as of fibers that escaped the primary insult. It also triggers a systemic response of autoimmune T cells to MBP that might affect the course of degeneration of the injured nerve. Whether the effect of these T cells on the nerve is detrimental or beneficial may depend, in part, on the nature and level of the co-stimulatory molecules expressed by the damaged tissue.

[0191] Several co-stimulatory molecules have recently been identified, including the B7 and CD40 molecules (Caux et al, 1994; Lenschow et al, 1996). CD40 appears to be dominant during cell differentiation in the lymph nodes and B7 during activation of T cells in the target organ (Grewal et al, 1996).

[0192] The B7 co-stimulatory molecule is a member of the immunoglobulin superfamily that interacts with CD28 and CTLA-4 on T_H cells. There are two related forms of B7 (B7.1 and B7.2). Both molecules have a similar organization of extracellular domains but markedly different cytosolic domains. Both B7 molecules are expressed on antigen-presenting cells (APCs) such as dendritic cells, activated macrophages and activated B cells as B7.1 or B7.2., which might preferentially support activation of the Th1 or the Th2 type of immune response, respectively (Kuchroo et al, 1995;

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Karandikar et al, 1998). We were therefore interested in determining the identity of B7 molecule subtype expressed in intact and injured CNS white matter, and its possible influence on the course of the response to the injury.

5   **RESULTS**

[0193] The co-stimulatory molecule expressed constitutively in the intact optic nerves of adult Lewis rats was identified as B7.2. (Figs. 12A, 12B). To examine the effects of neurotrauma on the expression of B7 co-stimulatory molecules, 10 we inflicted a mild crush injury on the optic nerves of Lewis rats and assessed the neural expression of B7 by immunohistochemical analysis. The most striking effect of the injury was seen on B7.2 expression manifested on post-injury day 3 by its elevation at the margins of the injury site 15 (Figs. 12C, D, E). In contrast, expression of B7.1 was not detected in the optic nerve either before or 3 days after injury. On day 7, however, B7.1 was detectable at the site of injury, having pattern reminiscent of that seen for macrophages or microglia (Fig. 12F).

20   [0194] Next, we attempted to determine whether the degenerative response to optic nerve injury could be modified by peripheral manipulation of the immune system. The manipulation chosen was induction of oral tolerance, known to cause a "bystander" T cell immunosuppressive effect (Weiner et

al, 1997b). Ingestion of low doses of MBP results in the activation of T cells which, based on antigen recognition, secrete TGF as the dominant cytokine and thus favor an immune response of Th2/3 type (Chen et al, 1994).

[0195] Lewis rats were fed with food to which 1 mg of bovine MBP had been added five times daily every other day. Ten days after first receiving the supplement, the rats were subjected to mild unilateral optic nerve crush injury. This time, interval between initiation of oral tolerance and injury was chosen to allow adequate build-up of the systemic T cell response. As shown in Figs. 13A and 13B, the numbers of macrophages or active microglia (indicated by ED-1 labeling) and T cells (indicated by immunolabeling for T cell receptor), assessed 3 days after injury, did not differ from those observed in control injured rats which did receive any treatment or were fed with PBS. In the rats with induced oral tolerance to MBP, however, the amounts of B7.2 were further increased at the margins of the site of injury (Fig. 13C) as compared with controls (Fig. 12E). In addition, in the rats with induced oral tolerance to MBP, B7.2 was also elevated at the site of injury relative to the control nerves (Fig. 13C). It seems reasonable to assume that the T cells exposed to MBP via intestinal absorption, upon invading the injured CNS,

contributed to the increase in expression of B7.2 by the injured nerve.

[0196] We then attempted to determine whether the observed changes in B7.2 expression in the injured rats was correlated with the extent of neuronal degeneration. Acute injury of the rat optic nerve is followed by a process of nerve degeneration, which can be quantified by retrograde labeling of the surviving neurons and counting of the corresponding cell bodies. Two weeks after optic nerve injury, the number of surviving RGCs, representing still-viable neurons, in the group of MBP-fed rats, was significantly higher than that in the control group, or that in the group of rats with injured nerves that were fed with ovalbumin (OVA). Interestingly, the benefit of the induced oral tolerance to MBP was increased by feeding the rats with more intensive schedule (Fig. 14).

EXAMPLE 5: POSTTRAUMATIC IMMUNIZATION WITH NOGO P472 PEPTIDE PROMOTES FUNCTIONAL RECOVERY FROM SPINAL CORD CONTUSION

MATERIALS AND METHODS

[0197] **Animals.** Female and male SPD rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science, Rehovot, Israel, matched for age (8-12 weeks) and housed in light- and temperature-controlled rooms.

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[0198] **Antigen.**   The Nogo p472 peptide (SEQ ID NO:19) was synthesized at the Weizmann Institute of Science, Rehovot, Israel.

[0199] **Immunization.**   Rats were immunized with 100-150 µg of Nogo p472 peptide emulsified in CFA containing 1 mg/ml *Mycobacterium tuberculosis.*   The emulsion was injected subcutaneously at one site in the upper back in the rats.   The p472-immunized rats were boosted one week after injury with p472 (100 µg/rat) in IFA.   Control rats were injected either with PBS or with PBS emulsified in CFA (Difco, Detroit, MI, USA).

[0200] **Contusion.**   Adult rats were anesthetized and their spinal cords were exposed by laminectomy at the level of T9. One hour after induction of anesthesia, a 10-g rod was dropped onto the laminectomized cord from a height of 25 mm or 50 mm, using the NYU impactor (Basso et al, 1995 and 1996).

Introduction

[0201] Regeneration of axons after injury in the CNS of higher vertebrates is extremely limited and almost absent. Growth inhibitors associated with CNS myelin play an important role in this aspect.   A potent neurite growth inhibitory activity associated with adult CNS oligodendrocytes and myelin was reported by Caroni and Schwab, 1988, and found to be neutralized by a monoclonal antibody, IN-1, which was shown to

promote axonal regeneration and to enhance compensatory

plasticity following spinal cord or brain lesions in adult

rats.

[0202] This activity was later related to a high molecular

5   weight membrane protein, designated NI-250, with a smaller

component, NI-35, in rat. The bovine homologue of rat NI-250,

bNI-220, was recently purified (Chen et al, 2000; PCT

Publication WO 00/31235). The cloning of *nogo* A, the rat cDNA

encoding NI-220/250, was recently reported (see Fig. 1a of

10   Chen et al, 2000; and PCT Publication WO 00/31235, the entire

contents of both of which being hereby incorporated herein by

reference). The rat *nogo* gene (SEQ ID NO: 17) encodes at

least three major protein products: Nogo-A (SEQ ID NO:18)

(1,163 amino acids; database accession number AJ242961),

15   Nogo-B (SEQ ID NO:20) (360 amino acids; AJ242962) and Nogo-C

(SEQ ID NO:21) (199 amino acids; AJ242963). The sequence of

the amino acid p472 (SEQ ID NO:19) containing the residues

623-640 of rat Nogo-A, is shown in the box in Fig. 1a of Chen

et al, 2000. The cloning of the corresponding human cDNA and

20   protein is reported in Prinjha et al, 2000. See also WO

00/60083 and WO 01/36631.

[0203] PCT Publication WO 00/31235 describes methods for

the production of recombinant Nogo proteins, fragments,

derivatives and analogs thereof, and DNA molecules coding

therefor. This publication further describes the use of a
Nogo protein or fragment thereof for the treatment of
neoplastic diseases of the CNS such as glioma, glioblastoma,
retinoblastoma, and the like; and further describes the use of
5   a ribozyme or an antisense Nogo nucleic acid for treatment of
a subject with damage to the CNS and/or for inducing
regeneration of neurons, wherein said ribozyme or antisense
Nogo nucleic acid acts by inhibiting the production of Nogo in
the subject. It is thus unexpected that immunization with
10 Nogo or fragments thereof and/or administration of T cells
activated therewith can promote nerve regeneration or prevent
or inhibit neuronal degeneration in the NS, as shown according
to the present invention.

[0204] PCT publication WO 00/31235, WO 00/60083 and WO
15 01/36631 are all hereby incorporated herein by reference. All
CNS protein polypeptides and nucleotides disclosed herein can
be used in the process of the present invention.

RESULTS

[0205] SPD male rats (n=5 per group) were subjected to
20 severe spinal cord contusion as described in the Materials and
Methods section of this example and a 10-g rod was dropped
onto the laminectomized cord from a height of 50 mm (Fig. 24A)
or 25 mm (Fig. 24B) using the NYU impactor. Soon thereafter
the rats were immunized subcutaneoulsy with Nogo p472 peptide

(100 µg/rat) emulsified in CFA containing 1 mg/ml

Mycobacterium tuberculosis. Control male rats (n=5 per group)

were injected with PBS emulsified in CFA.

[0206] In another experiment, 5 female rats were subjected

5   to severe spinal cord contusion as described in the Materials

and Methods section of this example and a 10-g rod was dropped

onto the laminectomized cord from a height of 50 mm (Fig. 25)

using the NYU impactor. Soon thereafter the rats were

immunized subcutaneoulsy with Nogo p472 peptide (100 µg/rat)

10   emulsified in CFA containing 1 mg/ml *Mycobacterium*

tuberculosis. Control female rats (n=5 per group) were

injected with PBS emulsified in CFA or with PBS alone. P472-

immunized rats were boosted one week after injury and

immunization with p472 (100 µg/rat) in IFA.

15   [0207] Acute incomplete spinal cord injury at the low

thoracic levels causes an immediate loss of hindlimb motor

activity that spontaneously recovers within the first 12 days

post-injury and stabilizes on deficient movement abilities.

The amount of motor function restoration is the sum up effect

20   of the positive recovery from spinal shock and the negative

effect of longitudinal and ventral spread of damage. A

therapeutic approach aiming at reducing the spread of damage

through neuroprotection will result in a better recovery in

terms of hindlimb motor activity. The hind limb motor skills

of the animals were scored using the BBB scoring method
developed by Basso et al, 1996, following the kinetics and
amount of hindlimb motor activity in the two experimental
groups.

5   [0208] The results of the experiments above, depicted in
Figs. 24 (A-B) and 25, show that both female (squares, Fig.
25) and male (triangles, Figs. 24 A-B) p472-immunized rats
showed significantly improved overall functional recovery
compared to the control rats injected with PBS in CFA
10   (squares, Figs. 24 A-B and circles, Fig. 25) or PBS only
(triangles, Fig. 25) (P≤0.01, one way repeated measurements
ANOVA). Males (Figs. 24 A-B) showed significantly better
locomotor performance than PBS+CFA-treated controls from day
11 after the injury and at all times points measured
15   thereafter. Females (Fig. 25) showed significantly better
hind limb locomotion than PBS-treated controls from day 25 and
on ((P≤0.05, **P≤0.01, ***P≤0.001, two-tail Student T-test).

Discussion of Experimental Results

[0209] The results of the experiments described in Examples
20   1 and 2 show that activated T cells accumulate at a site of
injury in the CNS. Furthermore, the results also demonstrate
that the accumulation of T cells at the site of injury is a
non-specific process, i.e., T cells which accumulated at the
site of injury included both T cells which are activated by

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exposure to an antigen present at the site of injury as well as T cells which are activated by an antigen not normally present in the individual.

[0210] The results of experiments described in Example 3 demonstrate that the beneficial effects of T cells in ameliorating damage due to injury in the CNS are associated with an NS-specific self-antigen as illustrated by MBP. More specifically, the administration of non-recombinant T cells which were activated by exposure to an antigen which can cause autoimmune disease (T_{MBP}), rather than aggravating the injury, led to a significant degree of protection from secondary degeneration. Thus, activating T cells by exposure to a fragment of an NS-specific antigen was beneficial in limiting the spread of injury in the CNS. The present findings show that secondary degeneration can be inhibited by the transfer into the individual on non-recombinant T cells which recognize an NS-specific self antigen which is present at a site of injury. The T cells may recognize cryptic or non-pathogenic epitopes of NS-self antigens.

[0211] In addition, the experiments described in Examples 3, 4 and 5 show that activation of T cells by administering an immunogenic antigen (e.g., MBP) or immunogenic epitope of an antigen (e.g., MOG p35-55 or Nogo p472), may be used for

preventing or inhibiting secondary CNS degeneration following injury.

[0212] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation, and that other means or steps for carrying out the same function can be used; and it is intended that such expressions be given their broadest interpretation.

[0213] All publications cited herein are incorporated by reference in their entirety.

REFERENCES

Altschul, et al, 1990, J. Mol. Biol. 215(3):403-410.

Arquint et al, Proc. Natl. Acad. Sci. USA 84:600-604, 1987.

Ashwood-Smith, 1961, Nature 190:1204-1205.

Basso, DM, Beattie, MS and Bresnahan, JC, 1995, "A sensitive
    and reliable locomotor rating scale for open field
    testing in rats, J. Neurotrauma 12(1):1-21.

Basso DM, Beattie, MS and Bresnahan, JC, 1996, "Graded
    histological and locomotor outcomes after spinal cord
    contusion using the NYU weight-drop device versus
    transection, Exp.Neurol. 139(2): 244-256.

Bazan, N.G., Rodriguez de Turco, E.B., Allan, G., 1995,
    "Mediators of injury in neurotrauma: intracellular signal
    transduction and gene expression", J. Neurotrauma 12:791-
    814.

Bender et al, 1960, J. Appl. Physiol. 15:520.

Ben-Nun, A., et al, 1981a, "The rapid isolation of clonable
    antigen-specific T-lymphocyte lines capable of mediating
    autoimmune encephalomyelitis Eur. J. Immunol. 11:195-199.

Ben-Nun, A., Wekerle, H. and Cohen, I.R., 1981, "Vaccination
    against autoimmune encephalomyelitis with T-lymphocyte
    line cells reactive against myelin basic protein", Nature
    292:60-61.

Ben-Nun, A. and Cohen, I.R., 1982, "Experimental autoimmune
    encephalomyelitis (EAE) mediated by T-cell lines: process
    of selection of lines and characterization of the cells",
    J. Immunol. 129:303-308.

Bone-Marrow Conservation, Culture and Transplantation,
    Proceedings of a Panel, Moscow, July 22-26, 1968,
    International Atomic Energy Agency, Vienna, pp. 107-186.

Brandt et al, 1992, J.Neurosci.Methods 45:35-40.

Burns, J., Rosenzweig, A., Zweiman, B., Lisak, R.P., 1983,
    Isolation of myelin basic protein-reactive T-cell lines
    from normal human blood", Cell Immunol. 81:435-440.

Caroni, P. and Schwab, M.E., 1988, "Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading" J. Cell Biol. 106: 1281-1288.

Caux et al, 1994, "Activation of Human Dendritic Cells through CD40 Cross-Linking", J. Exp. Med. 180:1263-1272.

Chen, Y., Kuchroo, V.K.,. Inobe, J. Hafler, D.A. & Weiner, H.L., 1994, "Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis" Science 265:1237-1240.

Chen, M.S., Huber, A.B., van der Haar, M.E., Schwab, M.E., 2000, "Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1", Nature 403:434-439.

Cohen, I.R., 1992, Immunol. Today 13, 490-494.

Diehl et al, Proc. Natl. Acad Sci. U.S.A. 83(24):9807-9811, 1986 (published erratum appears in Proc Natl Acad Sci U.S.A. 86(6):617-8, 1991).

Duvdevani et al, 1990, Neurol. Neurosci. 2:31-38.

Elias et al, 1991, Proc. Natl. Acad. Sci. USA 88:3088-3091.

Faden, A.I., et al, 1992, Trends Pharmacol. Sci. 13:29-35.

Faden, A.I., 1993, "Experimental neurobiology of central nervous system trauma", Crit. Rev. Neurobiol. 7:175-186.

Fournier, A.E., GrandPré, T., Strittmatter, S.M., 2001, "Identification of a receptor mediating Nogo-66 inhibition   of axonal regeneration", Nature 409:341.

Gonzalez et al, Mol. Phylogent. Evol. 6:63-71, 1996.

Gorin, Clinics in Haematology, 1986, 15(1):19-48.

Grewal et al, 1996, "Requirement for CD40 Ligand in Co-stimulation Induction, T Cell Activation, and Experimental Allergic Encephalomyelitis", Science 273:1864-1867.

Hauben, E. et al, 2000, "Passive or active immunization with myelin basic protein promotes recovery from spinal cord contusion", J. Neurosci. 20:6421-6430.

Hickey, W.F. et al, 1991, "T-lymphocyte entry into the central nervous system", J. Neurosci. Res. 28:254-260.

Higgins, et al, 1996, Methods Enzymol 266:383-402.

Hirschberg, D.L., et al, 1998, "Accumulation of passively transferred primed T cells independently of their antigen specificity following central nervous system trauma", J. Neuroimmunol. 89:88-96.

Hirshfeld, et al, 1970, FEBS Lett. 7:317.

Hovda, D.A. et al, 1991, Brain Res. 567:1-10.

Hunig et al, 1989, "A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. Differential reactivity with subsets of immature and mature T lymphocytes", J. Exp. Med., 169:73-86.

Hutchinson, C., et al, 1978, J. Biol. Chem 253:6551.

Janeway, C.A. Jr., 1992, "The immune system evolved to discriminate infectious nonself from noninfectious self", Immunol. Today 13:11-16.

Kamholz et al, 1986, Proc. Natl. Acad. Sci. U.S.A. 83(13):4962-4966.

Karandikar et al, 1998, "Targeting the B7/CD28:CTLA-4 co-stimulatory system in CNS autoimmune disease", J. Neuroimmunol. 89:10-18.

Kerschensteiner, M. et al, 1999, J. Exp. Med. 189:865-870.

Kramer, R. et al, 1995, Nature Med. 1(11):1162-1166

Kuchroo et al, 1995, "B7-1 and B7-2 co-stimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy", Cell 80:707-718.

Lazarov Spiegler, O., et al, 1996, FASEB J. 19:1296-1302.

Lenschow et al, 1996, "CD28/B7 System of T Cell co-stimulation", Annu. Rev. Immunol. 14:233-258.

Lewis et al, 1967, Transfusion 7(1):17-32.

Linner et al, J. Histochem. Cytochem., 1986, 34(9):1123-1135.

Livesey and Linner, Nature , 1987, 327:255.

Lovelock, Biochem. J. 56:265, 1954.

Lovelock and Bishop, Nature 183:1394-1395, 1959.

Lynch, D.R. et al, 1994, "Secondary mechanisms in neuronal trauma", Curr. Opin. Neurol. 7:510-516.

Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Martin, R. et al, 1990, J. Immunol. 145:540-548.

Martin, R. 1997, J. Neural Transm. Suppl. 49:53-67.

Mazur, Science, 1970, 168:939-949.

McIntosh, T.K., 1993, :Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review", J. Neurotrauma 10:215-261.

Moalem, G. et al, 1999, "Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy", Nature Med. 5: 49-55.

Mor et al, 1990, " Clinical modeling of T cell vaccination against autoimmune diseases in rats. Selection of antigen-specific T cells using a mitogen", Clin. Invest. 85:1594-1598.

Mor et al, 1995, "Pathogenicity of T cells responsive to diverse cryptic epitopes of myelin basic protein in the Lewis rat", J. Immunol. 155:3693-3699.

Nave et al, Proc. Natl. Acad. Sci. U.S.A 84:600-604, 1987.

Ota, K. et al, 1990, "T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis", Nature 346:183-187.

Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444-8.

Pette, M. et al, 1990, "Myelin basic protein-specific T lymphocytes lines from MS patients and healthy individuals", Proc. Natl. Acad. Sci. USA 87:7968-7972.

Phan The Tran and Bender, 1960, <u>Proc. Soc. Exp. Biol. Med.</u>
104:388.

Phan The Tran and Bender, <u>Exp. Cell Res.</u> 20:651, 1960.

Phan The Tran and Bender, 1961, in <u>Radiobiology, Proceedings
of the Third Australian Conference on Radiobiology,</u>
Ilbery, P.L.T., ed., Butterworth, London, p. 59.

Popovich, P.G., Stokes, B.T., Whitacre, D.C., 1996, "Concept
of autoimmunity following spinal cord injury: possible
roles for T lymphocytes in the traumatized central
nervous system", <u>J. Neurosci. Res.</u> 45:349-63.

Popovich et al, 1997, <u>J. Comp. Neurol.</u> 377:443-464.

Prinja et al, "Inhibitor of neurite outgrowth in humans",
<u>Nature</u> 403(6768):383-384 (2000)

Rapalino, O., Lazarov-Spiegler, O., Agranov, E., Schwartz, M.,
1998, "Implantation of stimulated homologous macrophages
results in partial recovery of paraplegic rats", <u>Nature
Med.</u> 4:814-821.

Rapatz et al, <u>Cryobiology</u> , 1968, 5(1):18-25.

Rinfret, <u>Ann. N.Y. Acad. Sci.</u> 85:576, 1960.

Roth et al, <u>Genomics</u> 28(2):241-250, 1995.

Rowe, <u>Cryobiology</u> , 1966, 3(1):12-18.

Rowe and Rinfret, 1962, <u>Blood</u> , 20:636.

Rowe et al, <u>Fed. Proc.</u> , 1962, 21:157.

Schaich et al, 1986, <u>Biol. Chem.</u> 367:825-834.

Schluesener, H.J. and Wekerle, H., 1985, " Autoaggressive T
lymphocyte lines recognizing the encephatoligenic region
of myelin basic protein: in vitro selection from unprimed
rat T lymphocyte populations", <u>J. Immunol.</u> 135:3128-
3133.

Sloviter and Ravdin, <u>Nature</u> 196:548, 1962.

Spitzer et al, <u>Cancer</u> , 1980, 45:3075-3085.

Stiff et al, <u>Cryobiology</u> , 1983, 20:17-24.

Streilein, J.W., 1993, Curr. Opin. Immunol. 5:428-423.

Streilein, J.W., 1995, Science 270:1158-1159.

Suruhan-Dires Keneli et al, 1993 Euro. J. Immunol. 23:530.

Thompson, et al, 1994, Nucleic Acids Res. 22(22):4673-80.

Vergelli, M. et al, 1996, "Differential activation of human autoreactive T cell clones by altered peptide ligands derived from myelin basic protein peptide (87-99)", Eur. J. Immunol. 26: 2624-2634.

Weiner et al, 1997a, Annu. Rev. Med. 48:341-51.

Weiner et al, 1997b, "Tolerance Immune Mechanisms and Treatment of Autoimmune Diseases", Immunol. Today 18:335-343. Werkele, H., 1993, In The Blood-Brain Barrier, Pardridge, Ed., Raven Press, Ltd. New York, 67-85.

Wu, D. et al, 1994, J.Neurochem. 62:37-44.

Yoles, E. et al, 1992, Invest. Ophthalmol. Vis. Sci. 33:3586-3591).

Yoles et al, 1996, J. Neurotrauma 13:49-57.

Yoshina, A. et al, 1991 Brain Res. 561:106-119.

Zaroulis and Leiderman, Cryobiology 17:311-317, 1980.

Zivin, J.A., et al, 1991 Sci. Am. 265:56-63.